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Estimation of Selection Differentials from Fish Scales: A Step Towards Evaluating Genetic Alteration of Fish Size in Exploited Populations

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Miller, L.M., and A.R. Kapuscinski. 1994. Estimation of selection differentials from fish scales: a step towards evaluating genetic alteration of fish size in exploited populations. Can. J. Fish. Aquat. Sci. 51: 774–783.

We developed a model to predict genetic response to selection imposed by a size-selective fishery. Use of our model provides estimates of selection as a function of heritability and overall selection differential imposed against a size-related trait, length of a scale radius from the focus to a given annulus (radius *n*). Selection differentials were estimated using data from the walleye (*Stizostedion vitreum*) fishery in Red Lakes, Minnesota. Overall selection differentials, incorporating selection differentials estimated by age and sex, and weighted by the contribution to spawning, were negative for the 1955 spawning population and positive for that of 1958. Directions of selection differentials, estimated by age and sex, generally corresponded to expected directions of selection based on the shape of the gillnet selectivity curve for walleye, suggesting that scale radii are useful for estimating selection against size traits. Results indicated that selective effects of gear may be surprisingly complex and population characteristics must be well understood before changes in fishery management are considered. We suggest that measurement of overall selection differentials alone may provide a useful management tool for indicating genetic risks of selective fisheries.

Nous avons mis au point un modèle pour prévoir l'ajustement génétique à la sélection imposée par une pêche sélective par taille du poisson. Notre modèle conduit à des estimations de la sélection en fonction de l'héritabilité et d'un différentiel global de sélection qui s'exerce sur un trait qui est fonction de la taille, soit la longueur du rayon de l'écaille mesuré entre le foyer de l'écaille et un annulus donné (rayon *n*). Des différentiels de sélection ont été estimés à partir de données sur la pêche au doré (*Stizostedion vitreum*) des lacs Red au Minnesota. Les différentiels globaux de sélection, qui comprenaient ceux estimés par âge et par sexe, et pondérés par la contribution à la ponte, ont été négatifs pour la population de géniteurs de 1955 et positifs pour celle de 1958. Le sens pris par les différentiels de sélection, par âge et par sexe, correspond généralement aux résultats que laissait entrevoir la forme de la courbe de sélectivité du filet maillant dans le cas du doré; cela pourrait indiquer que la mesure du rayon de l'écaille constitue un moyen utile d'estimer la sélection qui s'exerce contre des traits associés à la taille. Les résultats ont indiqué que les effets de la sélection des engins de pêche peuvent être étonnamment complexes et qu'il faut bien comprendre les caractéristiques démographiques avant d'envisager d'apporter des modifications à la gestion d'une pêche. Nous avançons l'hypothèse à l'effet que la mesure des différentiels globaux de sélection en soi peut constituer un instrument utile de gestion qui sert à indiquer les risques sur le plan génétique des pêches sélectives.

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Miller (1957) first questioned whether genetic patterns of fishes have been altered by selective fishing. He concluded that there was no clear-cut evidence of exploitation causing heritable changes. Since then, the idea of genetic changes resulting from harvest has arisen many times in the literature. Most often it has been proposed that a fishery may select against faster growth due to preferential removal of faster growing individuals (e.g., Handford et al. 1977; Ricker et al. 1978; Kirpichnikov 1981; Wohlfarth 1986; Parma and Deriso 1990). Beverton et al. (1984) suggested that fisheries whose gear is not in some way selective for large and faster growing fish are probably the exception rather than the rule.

Others acknowledged the potential of fisheries to alter genetic composition but questioned whether it necessarily leads to decreased growth and whether any type of change has ever been conclusively demonstrated. Beverton et al. (1984) discussed that, in some cases, when a cohort has entered the exploited phase, those that are fastest growing may become progressively less vulnerable. For example, Atlantic cod (*Gadus morhua*), which are most heavily fished in their immature phase, showed increased growth rapidly after exploitation (ICES 1983). Nelson and Soulé (1987) found few good examples of intrapopulation selection against faster or slower growth rates. Many claims had to be discounted because they just showed declines in average age or

size at maturity without additional evidence such as growth rate changes to indicate that the declines were not due to nongenetic responses to exploitation.

Concerns that a fishery may genetically select against certain size-classes are twofold: first, alteration of fish size may affect the economic or recreational value of the fish resource to humans, and second, directional selection lessens genetic variability (Hershberger 1988; Falconer 1989), which may lead to reduced population fitness. Selection imposed on size traits may affect these traits directly or may result in correlated responses of other traits due to the importance of size to many life-history characteristics (e.g., metabolism, fecundity, maturation, habitat use, feeding, and reproductive behavior).

Allendorf et al. (1987) suggested that all populations of fish included in a fishery will inevitably be genetically changed by harvesting. They recognized, however, the difficulty in proving genetic causes of changes in natural populations. Three factors were identified as being necessary to predict genetic changes: (1) the pattern and magnitude of selection imposed on fish with different traits, (2) the underlying genetic basis of traits, and (3) the phenotypic and genotypic correlations between traits.

In this paper, we present a method to quantify the first factor by estimating selection differentials imposed by a selective fishery on size traits, specifically on scale dimensions, which are indicators of size. We suggest that measuring the presence of selection differentials alone may be a useful genetic management tool, providing that reasonable assumptions can be made about the remaining two factors, if managers wish to predict the magnitude of potential genetic impacts of a selective fishery.

Methods

Model Derivation

The model is based on a fundamental principle of quantitative genetics, response to selection. Response to selection (R) is a change in the population mean value of a trait in response to selective forces. It is defined as the difference in mean phenotypic value between the offspring of selected parents and the whole parental generation before selection (Falconer 1989). The expected response to one generation of selection is the product of the selection differential (S) and heritability in the narrow sense (h^2) of the trait:

$$(1)\quad R = h^2 S$$

where h^2 ranges in value from 0 to 1.0.

The selection differential is the average superiority of the selected parents relative to the entire parental generation. It is measured as the difference between the mean value of the parents selected for breeding and the mean of all individuals in the parental generation before selection occurred.

Heritability, in the narrow sense, is a quantitative measure of genetic determinants of resemblance between relatives. It describes the contribution of additive genetic effects to the phenotypic variance of a trait in a population (Kapuscinski and Jacobson 1987; Falconer 1989).

Overall selection differential, S_o

Measurement of selection differentials must account for multiple age-classes that occur in spawning populations of most fish species. For a given spawning season, our model estimates selection differentials separately for each existing age-class and weights them (as explained below) to form an overall selection differential (S_o). Fish of different age-classes have experienced different environmental conditions throughout their growth history; comparisons of growth between age-classes are therefore confounded by environmental effects. Because fish within an age-class have experienced essentially the same broad environmental conditions, we assumed that size differences found among individuals indicate genetic growth differences in a common environment. Selection differentials within an age-class are also estimated separately for males and females because a different weighting factor is used for each sex for reasons explained below.

The equation for the overall selection differential, imposed in a given spawning season, as a function of sex and age-class is

$$(2)\quad S_o = [\Sigma(S_{x,f}\, W_{x,f}) + \Sigma(S_{x,m}\, W_{x,m})]/2$$

where S_o = overall selection differential, Σ = summation over all age classes of spawning fish, S = selection differential, x = age at spawning, m and f = sex indicators, male or female, and W = weighting factor. Weighted selection differentials are summed for each sex and averaged to give an overall selection differential imposed on a spawning population.

Modified equation for response to selection

Substituting S_o for S, equation 1 becomes

$$(3)\quad R = h^2 S_o.$$

This predictive equation for the expected change in mean value of a trait (R) as a function of heritability (h^2) and the overall selection differential (S_o) relies on two assumptions: (1) matings between males and females of different ages are random and (2) survival of embryos is independent of their parents' ages. Violation of assumption 1 would not be critical unless assortative mating resulted in a substantial fraction of mature fish not spawning because suitable mates were unavailable. If assumption 2 is violated, an estimate of the relationship between survival and age of parents could be used to adjust weighting factors.

Weighting factors — Each weighting factor accounts for the proportion of all progeny contributed by an age-class within the spawning population. This is estimated differently for each sex.

The proportion of all progeny contributed by an age-class of females (W_f) is estimated as the proportional contribution of that age-class to all available eggs:

$$(4)\quad W_f = n_x \cdot \mathrm{fec}_x / \Sigma(n_x \cdot \mathrm{fec}_x)$$

where x = age, n = number of spawning females, fec = fecundity of a female of average length, and Σ = summation over all ages in the spawning population. Two phenomena are reflected in this weighting factor: (1) due to mortality and fluctuations in age-class strength, each age-class present at spawning represents a different proportion of the total spawners and (2) fecundity is positively correlated with fish size. This weighting factor requires the simplifying assumption that fecundity is linearly proportional to the actual number of embryos produced. If available data suggested a nonlinear relationship between fecundity and actual numbers of embryos produced, the weighting factor could be adjusted accordingly.

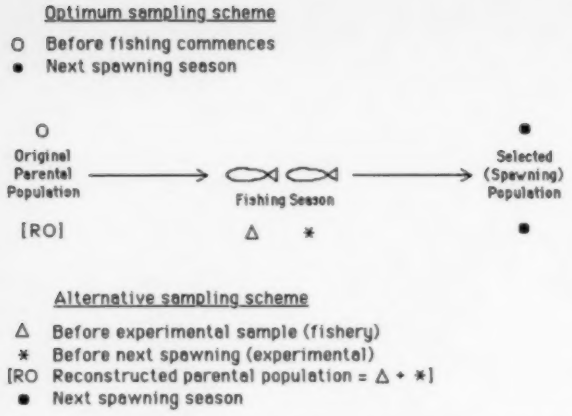

FIG. 1. Schematic diagram of optimum sampling scheme for measuring selection differentials and alternative scheme if the frequency distribution of a trait in the parental population must be reconstructed.

The weighting factor for males (W_m) is calculated as the proportion of all spawning males in an age-class:

$$(5)\quad W_m = n_x/\Sigma\, n_x$$

where x = age, n = number of spawning males, and Σ = summation over all ages at spawning. This assumes an equal progeny contribution across males regardless of age, although this factor could be adjusted if data were available suggesting other relationships.

Methods Associated with Application of the Model

This section first outlines general data requirements and then describes the specific fishery and associated data used to illustrate application of the model.

Data requirements

Use of scale radii — To apply the model to natural populations, we have used the length of a scale radius from the focus to a specified annulus as the selected size trait. Measuring selection against size traits in a mixed-age spawning population is complicated by the correlation between size and age; because fish continue to grow throughout most of their lives, direct comparisons of fish of different ages would confound growth and age effects. A typical practitioner of a breeding program has individual performance records so that all individuals can be compared on the basis of a common trait, such as size at 1 yr of age. Few natural populations allow us to obtain individual performance records on size traits. Fortunately, individual fish contain such performance records in marks laid down in bony structures.

Marks in bony structures have often been used as indicators of fish size (Carlander 1987). Any structure with measurable annual marks could be used, such as otoliths or spines. We used scales because of their availability in an extensive historical collection and because they are routinely collected by fishery managers.

Use of scale radii allows us to compare fish of different ages for a common growth trait, where the common trait is scale radius at an earlier age. Any radius up to the first age that fish are recruited into the fishery may be used. Selection against a common radius at an age after recruitment cannot be estimated because the younger age-classes exposed to the fishery have not yet expressed the trait. The term radius means the length (in millimetres) from the scale focus to a specific annulus; for example, radius 2 is the length from the scale focus to its second annulus.

Use of radius lengths at an earlier age to assess selection imposed by a fishery requires that these data correlate with fish sizes at later ages when the fishery acts. Many estimates of positive correlations between individual fish size at two ages have been reported (Tave 1986). Making the common assumption that scale growth is positively correlated with fish growth (Van Oosten 1929; Hile 1970), radius lengths laid down at earlier ages should also positively correlate with fish size at later ages. Our data for illustration of the model met this assumption in most cases, as explained below in the results section.

Sampling for scale measurements — Calculating selection differentials requires estimates of mean scale radius lengths of mature fish at two times in a year, before fish are selectively removed and at the following spawning season (Fig. 1, optimum sampling scheme). In the language of selective breeding, the population present before fishing is the parental population and those surviving to spawn after fishing make up the selected population (Kapuscinski and Jacobson 1987). First, mean scale radius lengths are calculated for each sex and age separately. Then, $S_{x,f}$ and $S_{x,m}$ are estimated by calculating the differences between the spawning population means and the original parental population means.

Sampling for weighting factors — Weighting factors require estimates calculated from data other than measurements of the selected trait. Both weighting factors require an estimate of the age distribution at spawning, which can be determined from fish sampled for scale radius measurements. The weighting factor for females also requires a regression of fecundity on size. This regression can be constructed from measurements of spawning fish size and counts of number of eggs per individual. Mean size of females at a given age is entered into the regression equation to estimate average fecundity in that age-class. Mean size of females can be determined from measurements routinely taken when scale samples are collected from managed populations.

Red Lakes fishery

Application of the model was illustrated using information from Red Lakes in north-central Minnesota. A commercial fishery for walleye has existed there since the early 1900's. Scales and growth measurements were taken from samples of fish collected from spawning runs, commercial harvest, and 3.8- to 10.2-cm-mesh (stretch measure) experimental gill nets during the years 1949–58 as described by Smith and Pycha (1961). The commercial fishery used 8.9-cm-mesh gill nets only. We randomly subsampled scales collected during the 1954 and 1957 summer experimental and commercial fishing and the 1955 and 1958 spawning runs. These years were chosen because the greatest number of scales was taken then.

Reconstructing original parental population — The random sampling conducted by Smith and Pycha (1961) occurred during the fishing season; therefore, it was necessary to reconstruct the frequency distribution of scale radius lengths in the parental population before fishing began. Reconstruction allowed estimation of the impact of the full fishing

season. Fishery samples were combined with the random samples based on an estimate of the proportion of the entire population removed by the fishery (Fig. 1, alternative sampling scheme). Mean scale radius estimates of these combined samples were used as estimates of parental population means when calculating selection differentials. For the 1954 fishing season, we combined data from a large experimental sample collected during August with June and July commercial fishing samples. To estimate selection differentials in the 1957 parental population, we combined data from a July experimental sample with June commercial data.

Because no estimates of exploitation for the years we examined were available, we considered a range of possible rates. An exploitation rate of 41% was estimated for the 1989 fishing season (Bushong 1990). Smith and Pycha (1961) estimated a total mortality rate of 65% for the 1947 year-class of walleye when fully available to fishing gear in Red Lakes, and Ney (1978) gave a range of 5–30% for natural mortality rates of walleye. We used rates based on 30–60% annual exploitation and divided according to monthly catch rates recorded by the fishery (L.L. Smith, Department of Fisheries and Wildlife, University of Minnesota, St. Paul, Minn., unpublished data). Because different exploitation rates did not change the resulting direction of the predicted overall selection, all reported results are based on an original population distribution of scale radii reconstructed using an estimate of 40% annual exploitation.

Scale measurements — Scale radius measurements and age determinations were made by the usual criteria applied to ctenoid scales (Jearld 1983). Most of the samples used had been aged by Smith and Pycha (1961) who determined their aging to be reliable because year-class strength fluctuations could be followed through successive years, growth rates in a given year were similar when calculated from different years of data, and the extremely poor growth year of 1950 laid a distinctive band on many of the scales sampled in subsequent years.

Scale impressions were made on acetate slides and enlarged 41 times for measurement. Printed copies were made of two scales per fish on a Minolta wet-process microfiche reader-printer. Annuli were marked on the copies and measurements were made using a Houston Instruments Hipad digitizer and DisBcal (Frie 1982). Average radius (to the nearest 0.001 mm) of the two scales per individual was used in calculations, as recommended by Newman and Weisberg (1987).

Weighting factors — Weighting factors were calculated by estimating the age distribution at spawning from the same random samples taken to measure scale radii in the selected (spawning) population. Weighting factors for females also involved estimates of fecundity from mean lengths of females by age using an empirical regression formula derived from data presented by Bushong (1990): ln(number of eggs) = 3.8095 ln(total length) −12.41, $R^2 = 0.961$. Bushong combined fecundity data from Red Lakes and Lake of the Woods, also in northern Minnesota, to cover a broader range of fish sizes because no significant differences were found between the two sets of data.

Error estimation — We estimated error in S_o using the bootstrap resampling technique (Efron and Gong 1983). In brief, radius length data for each age and sex were sampled with replacement, $B = 500$ times. For each resampling event, S_o was calculated according to equation 2. Standard errors were then estimated as

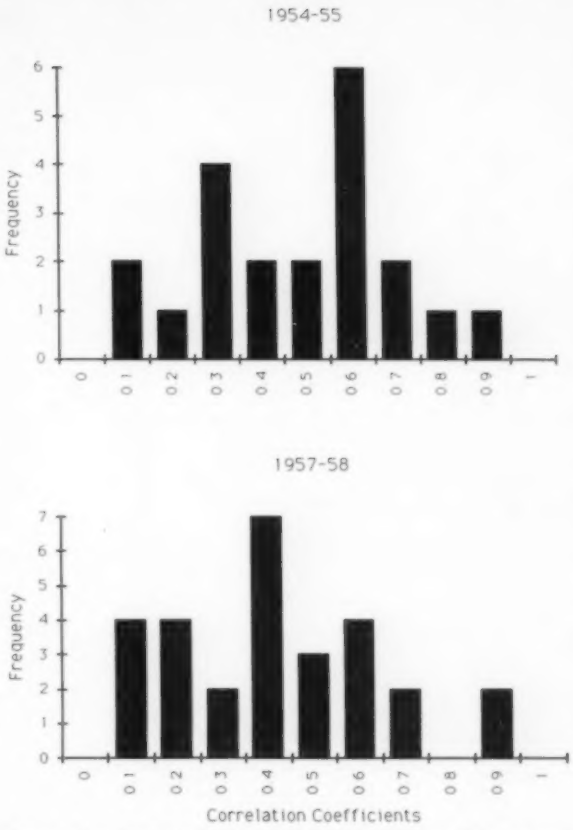

FIG. 2. Frequency histograms, for two sampling periods, of correlation coefficients between fish size at capture and length of radius 3. Within each sampling period, correlation coefficients were calculated for each data subset. Subsets consisted of fish of the same age and sex and sampled by the same gear type (commercial gill net, experimental gill net, or spawning trap nets).

$$\hat{\sigma} = [\sum(S_o^* - \bar{S}_o^*)^2)/(B - 1)]^{1/2}$$

where summation is over the 500 resampling events and the asterisk denotes a bootstrap estimate of S_o

Results

Results of applying our model to Red Lakes data are presented below. First, we outline results of correlation tests between radius length and size at capture. Next, estimated selection differentials are presented in two ways: by sex and age separately and by overall selection differentials. Finally, we present results using hypothetical data that explored impacts of changes in year-class strength and environmental conditions on our empirical estimates of overall selection differentials.

Correlations between Radius Length and Size at Capture

Our data generally met the assumption that radius length was positively correlated with fish size at capture (Fig. 2). Samples from 1954 and 1955 had average correlation coefficients between fish size at capture and length of radii 2 and 3 of 0.44 and 0.53, respectively. All coefficients, calculated by age-class, were positive, ranging from 0.13 to

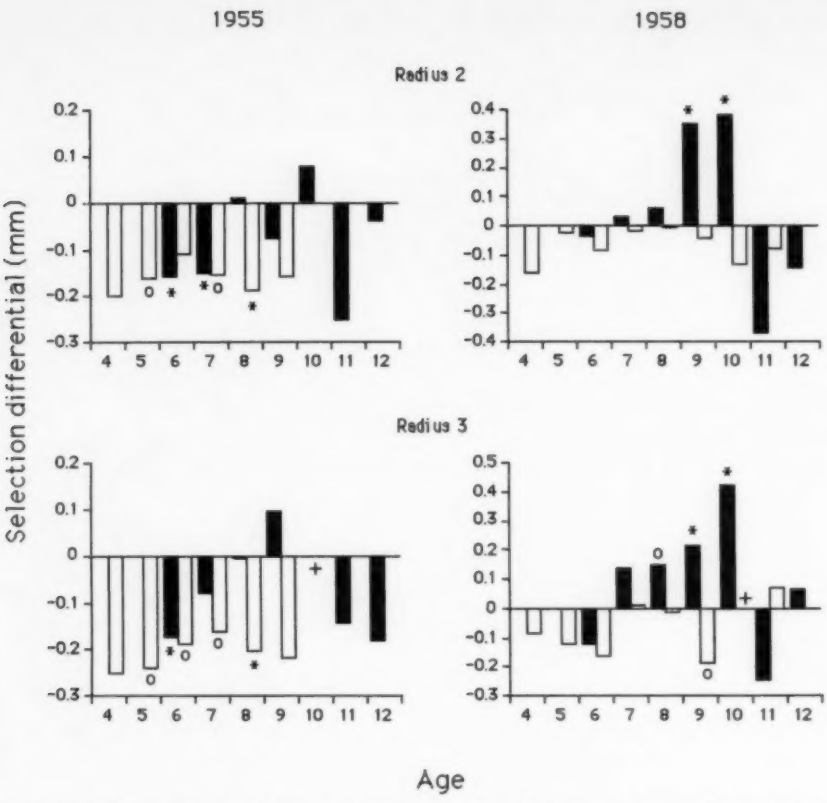

FIG. 3. Selection differentials by age and sex for length of radii 2 and 3 imposed on 1955 and 1958 spawning populations of walleye in Red Lakes, Minnesota. Open bars are estimates for males; solid bars are estimates for females. Significance levels (*t*-test for difference in mean radius length between spawning sample and reconstructed parental population sample): ($P < 0.05$) (asterisks) or ($P < 0.10$) (circles). For radius 3, estimated selection differentials (plus signs) were −0.002 mm for 1955 age 10 females and 0.001 mm for age 10 males, values too small to produce a visible bar on the vertical scale of these graphs.

0.92. Samples from 1957 and 1958 had lower average coefficients (0.30 and 0.43).

Selection Differentials by Age and Sex

Selection differentials (to the nearest 0.001 mm), estimated by age and sex, showed varying degrees of magnitude and differences in sign (Fig. 3; Appendix 1). Estimates of S_x, in standard deviation units of the original parental population, ranged in absolute value from 0.00 to 6.58 with a mean of 0.82 (Appendix 1). In 1955 (Fig. 3; Appendix 1A), females experienced negative selection differentials at the youngest and oldest ages and positive selection differentials at some intermediate ages. This shift to positive values occurred between ages 7 and 8 for radius 2, where mean size changed from 401.5 to 413.7 mm. For radius 3, however, the shift occurred between ages 8 and 9, but the unweighted selection differential for age 8 was barely negative (−0.003 mm). A return to negative values occurred at age 10 for radius 2 (mean size 525.7 mm) and age 11 for radius 3 (mean size 462.3 mm). Males of all ages experienced negative selection differentials for both radii.

Similar patterns were found in estimates for females in 1958, but males were not as consistent (Fig. 3; Appendix 1B). For females, the shift to positive values occurred between ages 7 and 8 (mean size changed from 399.1 to 418.5 mm). Estimates were again negative at age 11 (mean size 464.2 mm) but positive for radius 3 of age 12 females. Small sample size or incorrect assignment of age to older fish may have contributed to this apparent discrepancy in direction of selection for age 12. Results for males in 1958 were not as consistent as those in 1955. Radius 2 had negative values for all ages but radius 3 had three positive estimates.

Overall Selection Differentials

Overall selection differentials (Table 1) were estimated according to equation 2 using selection differentials by age and sex and weighting factors calculated as shown in Table 2. The model estimated overall negative selection differentials imposed on the 1955 spawning population for scale radii 2 and 3. Overall positive selection differentials were imposed on the 1958 spawning population for these traits.

Impacts of Age-class and Growth Patterns on Selection Differentials

Hypothetical data, based on the Red Lakes data, were used to illustrate the impacts of changing patterns of year-

TABLE 1. Estimates of overall selection differentials imposed on the 1955 and 1958 spawning populations of walleye in Red Lakes (standard error in parentheses).

Trait	Overall selection differential (mm)	
	1955	1958
Radius 2	−0.099 (0.026)	0.028 (0.027)
Radius 3	−0.111 (0.029)	0.042 (0.034)

class strength and growth on the magnitude and direction of selection. Table 3 shows the outcomes of several hypothetical changes. Cases A and B illustrate changes in the age-class distribution of females at spawning. In case A, age-class distribution was altered until the direction of selection shifted from positive to negative. The necessary changes resulted in a realistic distribution of ages, illustrating the dependence of the direction of overall selection on age-class distribution. Case B was created by shifting the numbers of fish in an age-class of 1955 females to one earlier age, thus altering age-class distribution. This yielded approximately 25% increases in the resulting overall selection differentials.

Case C reflects changes in growth rates. Each age-class of 1958 females was assigned the mean total length and resulting selection differential of the next older group, as if growth had been much better in the previous year. The overall selection differential for radius 2 increased, while for radius 3, it was virtually unchanged.

Discussion

To complete the model's prediction of response to selection (equation 3), an estimate of heritability is needed. Heritability is population and time specific. Estimates are generally made under controlled conditions where environmental components are stabilized or measurable and all members of the population can be measured. Because this is not the case in natural fish populations, it is generally unreasonable for a manager to estimate this component of the model for all populations. It may be appropriate, instead, to use a range of values based on heritability estimates for other populations.

Several potential sources of heritability estimates for size traits exist. One source is the increasing number of estimates from the aquaculture literature, which commonly reports heritability estimates for length and weight from 0.10 to 0.40 (Tave 1986). To estimate heritability in natural populations, which is likely to be lower due to increased environmental variance, an experimental approach as suggested by McAllister et al. (1992) may be useful. They found that a design including random and selective sampling of several populations should give sufficient power to estimate heritability with reasonable precision. Finally, if long-term sampling allows estimation of changes in the measured trait, then equation 3 can be rearranged to estimate heritability as $h^2 = S/R$.

S_o as a Genetic Risk Management Tool

Although the full model provides a predictive equation for estimating response to selection (equation 3), we suggest that estimating selection differentials alone (equation 2) may provide a useful management tool. Assuming that size traits (scale radius lengths in our case) are heritable in fish, risk of genetic alteration due to selection is indicated if the overall selection differential is nonzero. Continual imposition of directional selection may erode genetic variability that, in turn, may reduce a population's potential to evolve in the face of environmental change. Degree of risk may be roughly assessed by considering risk of genetic alteration to increase as the magnitude of S_o increases.

The above argument indicates the value of estimating heritability in the studied population or determining a likely range of values for natural populations. Alternatives to currently used selective gear may have great economic and biological impacts on fisheries. It is important to get a quantitative as well as qualitative assessment of risks before any management actions are taken to change uses of selective gear. However, if no selection differentials are imposed (and no stabilizing or disruptive selection is indicated (Herschberger 1988)), the trait cannot be genetically altered due to selection, regardless of its level of heritability, and no intervention is required.

Fisheries managers may be tempted to use the model in another way, to intentionally select a desired trait, for example, as suggested by Larkin (1981) and McAllister et al. (1992). Although this may be a reasonable alternative in captive aquaculture operations, it should generally be avoided

TABLE 2. Calculation of weighting factors (W_f for females and W_m for males) used to estimate overall selection differentials imposed on (A) 1955 and (B) 1958 spawning populations of walleye in Red Lakes. n_x = number of spawning fish of age x; Mean tl = mean total length of fish (mm); fec_x = number of eggs per female of mean size at age x; $W_f = n_x \cdot \text{fec}_x / \Sigma(n_x \cdot \text{fec}_x)$; $W_m = n_x / \Sigma n_x$.

	Females				Males	
Age (x)	n_x	Mean tl (mm)	fec_x[a]	W_f	n_x	W_m
			(A) 1955			
4					3	0.02
5					10	0.07
6	29	391.7	30779	0.14	71	0.53
7	32	401.5	33818	0.17	16	0.12
8	75	413.7	37902	0.44	27	0.20
9	26	440.5	48141	0.19	7	0.05
10	1	462.3	57867	0.01		
11	1	525.7	94418	0.01		
12	3	464.8	59068	0.03		
14	1	508.0	82869	0.01		
			(B) 1958			
4					6	0.05
5					26	0.21
6	8	395.3	31833	0.06	20	0.16
7	17	399.1	33017	0.12	29	0.23
8	41	418.5	39571	0.36	24	0.19
9	17	421.5	40651	0.15	11	0.09
10	12	456.7	55192	0.15	6	0.05
11	4	464.2	58724	0.05	4	0.03
12	6	515.0	87217	0.12		

[a] $\ln(\text{eggs}) = 3.8095 \ln(\text{total length}) - 12.410$, $R_2 = 0.961$.

TABLE 3. Case A: Hypothetical changes in age-class distribution of spawning females in 1958 that resulted in changed direction of overall selection differential (S_o) compared with empirical estimates for the real population in Red Lakes. Case B: Hypothetical changes in age-class distribution of spawning females in 1955 that resulted in more strongly negative values for overall selection differential compared with empirical estimates for the real population in Red Lakes (numbers of fish in an age-class were shifted to one earlier age to alter age distribution). Case C: Hypothetical changes in growth of spawning females in 1958 resulting in various degrees of change in the value of overall selection differential compared with empirical estimates for the real population in Red Lakes (to simulate greater growth rates, each age-class was assigned the mean total length of the next oldest age-class).

	Age distribution			S_o (mm)	
Age	Real	Hypothetical		Real	Hypothetical
			Case A		
6	0.08	0.18	Radius 2	0.028	−0.015
7	0.16	0.33	Radius 3	0.042	−0.002
8	0.39	0.18			
9	0.16	0.13			
10	0.11	0.04			
11	0.04	0.11			
12	0.06	0.04			
			Case B		
6	0.17	0.23	Radius 2	−0.099	−0.125
7	0.19	0.54	Radius 3	−0.111	−0.138
8	0.45	0.19			
9	0.15	0.01			
10	0.01	0.01			
11	0.01	0.02			
12	0.02	0.01			
14	0.01	0.01			
			Case C		
6	395.3	399.1	Radius 2	0.028	0.041
7	399.1	418.5	Radius 3	0.042	0.041
8	418.5	421.5			
9	421.5	456.7			
10	456.7	464.2			
11	464.2	515.0			
12	515.0	525.0			

for natural populations. Directional selection may lessen genetic variability, thereby imposing risks to the sustainability of the population. This would be compounded if managers tried to impose selection in order to counteract previous directional selection. Furthermore, other important traits that are genetically correlated with size may be changed in undesirable directions or magnitudes. Short-term gains may be offset by irreversible, long-term consequences. Careful evaluation of priorities for an exploited population and of our abilities to monitor and mitigate negative genetic effects is necessary before such an approach is taken.

Relation of Selection Differentials to Gear Selectivity

An important assumption of our method is that scale radii are accurate indicators of the selective nature of the fishery. Our results suggest that this attribute holds true for the population studied. Estimates of selection differentials by age and sex were generally consistent with the modal shape of the selectivity curve of gill nets for walleye estimated by Hamley and Regier (1973). Figure 4 shows the correspondence between the shape of the gillnet selectivity curve and direction of genetic selection differentials. At younger ages, hence smaller average sizes, a larger fish in an age-class is more likely to be captured than a smaller fish. At a later time, mean size of the age-class passes a peak in the selectivity curve and larger fish are then more likely to escape the gear. Gear selectivity for larger (or smaller) sizes may impose genetic selection against large (or small) size. In size ranges covering regions of increasing slope on the selectivity curve, a negative selection differential is expected; when the slope is decreasing, a positive selection differential would be imposed.

In the years we examined, it appears that few males in the Red Lakes population passed the first peak of selectivity due to their slow growth. Only 1958 male spawners of ages 7, 10, and 11 had positive selection differentials. For age 10 and 11 males, this was probably due to their large size

(mean total lengths of 383.1 and 409.0 mm, respectively). A positive selection differential for age 7 males cannot be explained by the selectivity of gill nets.

Females, however, grew faster and were present at older ages. Several age-classes therefore passed the first peak of selectivity and some may have approached the increasing slope of the second mode. Selection differentials for females shifted from negative to positive values as the mean size of age-classes apparently passed the first selectivity curve peak. Estimates were again negative as mean size of age-classes approached the second region of increasing slope on the curve.

Factors Affecting Overall Selection Differential

Model results for the Red Lakes fishery show that gill nets can impose one of several possible patterns of selection: overall positive selection differentials, overall negative selection differentials, or negative and positive selection by age that could balance under certain conditions resulting in no overall selection differential. The hypothetical cases presented in the results reinforce this conclusion.

An important factor affecting the direction of the overall selection differential is relative numbers in each year-class (Table 3, Cases A and B). In general, year-classes of older fish are expected to have significantly fewer members than younger year-classes due to natural and fishing mortality. Walleye, however, are known for large year-class strength fluctuations (Ney 1978). Strong year-classes will have relatively large weighting factors associated with their age-class at each spawning. Such year-classes will strongly influence the direction of overall selection as they increase in size with age and pass through various regions on the selectivity curve, as discussed in the previous section.

Environmental conditions for growth also affect the overall direction of selection. In a year of good growth, for example, mean size at age will change more during the fishing season (Table 3, Case C). Depending on the relative abundance of year-classes, many young fish will grow into the range of vulnerability to the gear, while many older fish will outgrow selectivity peaks. Environmental conditions for growth will strongly influence the selective pressures to which a year-class is exposed. In fact, Todd and Larkin (1971) suggested that the direction of selection imposed on sockeye salmon (*Oncorhynchus nerka*) by gill nets might change because gear selectivity shifted each year with changes in the mean size of members of each cohort.

The simple cases we have presented emphasize the need for familiarity with all population characteristics — year-class strengths, growth, and environmental factors that affect them — before management actions can be taken to alleviate the potential for genetic alterations by selective harvest. The complex mixture of environmental and year-class strength effects makes it difficult to predict future directions of selection.

Use of Radius 1

We recommend measuring a radius at the oldest age possible before recruitment into the fishery and, in particular, avoiding use of radius 1. Results for radius 1 (data not shown) showed that selection differentials did not consistently follow the pattern of sign changes found for radii 2 and 3, that average correlations between fish size at capture and radius lengths were weakest (0.33 and 0.15), and that these correlations were often negative, as seen in 9 of 29 samples in 1957–58.

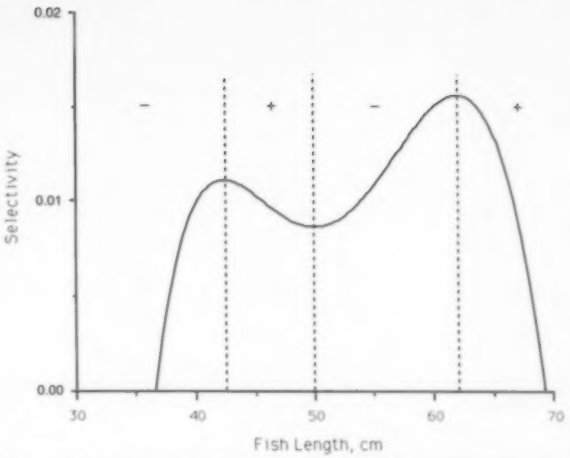

FIG. 4. Selectivity of 8.9-cm-mesh gill net for walleye (adapted from Hamley and Regier 1973). Plus and minus signs denote expected direction of the selection differential when mean total length of an age-class is in an indicated range.

The inconsistencies between radius 1 and other radii may have been caused by measurement error or biological factors. Inexact identification of the focus would affect radius 1 measurements proportionally greater than those of later radii. Smith and Pycha (1960) noted that first-year scale increments of age 1 walleye in Red Lakes were not closely correlated with those of older fish. Tave (1986) listed correlations between fish size at various ages that often showed the relationship to weaken as time between measurements increased. Because of these difficulties, first-year radii should be used cautiously unless their correlations with fish lengths are known to be consistent for the population studied.

Selective Removal of Immature Fish

In its present form the model estimates selection differentials for one spawning season but would not estimate the true long-term impacts of a fishery if a large portion of immature fish were selectively removed. In this situation, the original population, which is defined as mature fish present before the fishing season, would actually be a selected subset of all fish that would have matured if they were not vulnerable to the fishery. Therefore, to estimate selection differentials, the original population estimates must be taken from a sample of each year-class of mature fish when it was at an age before recruitment. To estimate the selection differential against radius 3 in 1955, for example, samples would be needed of the 1949 year-class in 1952, the 1948 year-class in 1951, etc.

Such an approach, however, has some weaknesses. Besides the inconveniences of obtaining a long period of samples, estimates of selection will include effects of natural selection and cumulative fishing selection over the increased number of years that pass between population estimates. Such additional effects may make it difficult to associate the selectivity of the gear with resulting selection differentials, as we were able to do with Red Lakes data.

Conclusion

We have presented a quantitative model and methods for its application to data routinely collected in the monitoring of fisheries. Application of this model provides a means of estimating genetic selection imposed by a fishery on natural fish populations. Measurement of selection differentials alone may provide a useful management tool for indicating genetic risks imposed by selective fishing, providing that size traits are shown to be heritable in natural populations. Our results from illustrative application of the model to one fishery indicate that selective effects of gear such as gill nets may be surprisingly complex and that population characteristics must be well understood before changes in management of the fishery are considered. Rowell et al. (1989) recognized the importance of measuring selection differentials, which they attempted to do using otolith samples from commercial trawl harvest. They were unsuccessful and indicated the need for a revised sampling scheme. Our method may fulfill this need.

Acknowledgments

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Appendix 1

Selection differentials by age and sex for length of radii 2 and 3 imposed on the (A) 1955 and (B) 1958 spawning populations of walleye in Red Lakes. S_2, S_3 = selection differentials (mm) for radii 2 and 3; sd units = selection differential in units of standard deviation from samples of the original parental population (S/sd). Significance levels (t-test for difference in mean radius length between spawning sample and reconstructed parental population sample): *$P < 0.05$; **$P < 0.10$.

Age (x)	S_2	sd units	S_3	sd units
		(A) 1955		
Females				
6	−0.159*	−0.97	−0.171*	−0.87
7	−0.149*	−0.75	−0.079	−0.32
8	0.012	0.05	−0.003	−0.01
9	−0.076	−0.42	0.096	0.41
10	−0.078	1.33	−0.002	−0.02
11	−0.250	−6.58	−0.144	−2.06
12	−0.037	−0.14	−0.179	−0.51
14[a]	0.000	0.00	0.000	0.00
Males				
4	−0.200	−2.55	−0.252	−2.90
5	−0.160**	−0.79	−0.239**	−0.91
6	−0.108	−0.46	−0.187**	−0.76
7	−0.152**	−0.70	−0.162**	−0.69
8	−0.186*	−0.81	−0.204*	−0.88
9	−0.158	−0.75	−0.218	−0.81
		(B) 1958		
Females				
6	−0.033	−0.13	−0.122	−0.31
7	0.030	0.16	0.139	0.51
8	0.063	0.43	0.146**	0.65
9	0.356*	1.61	0.214*	0.99
10	0.381*	1.76	0.424*	2.76
11	−0.368	−2.24	−0.243	−0.74
12	−0.140	−1.35	0.068	0.20
Males				
4	−0.161	−0.53	−0.086	−0.28
5	−0.021	−0.09	−0.120	−0.36
6	−0.081	−0.35	−0.162	−0.60
7	−0.017	−0.09	0.010	0.04
8	−0.005	−0.02	−0.010	−0.04
9	−0.041	−0.19	−0.186**	−0.72
10	−0.131	−0.50	0.001	0.00
11	−0.074	−0.35	0.069	0.21

[a]No age 13 females were present in the previous summer's experimental or commercial samples; therefore, no selection differentials were estimated.

Avoidance of Pentachlorophenol by Postlarval Brown Shrimp (*Penaeus aztecus*) (Decapoda, Penaeidae) in a Laminar-Flow Choice Chamber

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Benfield, M.C., and D.V. Aldrich. 1994. Avoidance of pentachlorophenol by postlarval brown shrimp (*Penaeus aztecus*) (Decapoda, Penaeidae) in a laminar-flow choice chamber. Can. J. Fish. Aquat. Sci. 51: 784–791.

Responses of postlarval brown shrimp (*Penaeus aztecus*) to pentachlorophenol (0–450 $\mu g \cdot L^{-1}$) were measured in synthetic seawater and estuarine water using a laminar-flow choice chamber. This chamber provides individual postlarvae with equal exposure to two parallel olfactant streams separated by a steep concentration gradient. Shrimp detected and avoided pentachlorophenol concentrations above 91 $\mu g \cdot L^{-1}$ in synthetic seawater. This detection threshold reflects limitations in statistical power, and with increased replication the physiological threshold could probably be resolved at a much lower concentration. Pentachlorophenol appeared to be more repellent when dissolved in estuarine water from Galveston Bay, Texas. The 96-h LC_{50} for pentachlorophenol was 317 $\mu g \cdot L^{-1}$ which suggests that postlarvae are capable of avoiding acutely toxic concentrations of this pollutant. For postlarvae of this species, behavioral avoidance appears to provide a more sensitive indicator of pollutant responses than the conventional toxicity bioassay.

La réaction de postlarves de la crevette brune (*Penaeus aztecus*) au pentachlorophénol (0–450 $\mu g \cdot L^{-1}$) a été mesurée dans une eau estuarienne et une eau de mer synthétique en circulation laminaire dans une enceinte à indication de décision. Cette enceinte permet d'exposer également des postlarves prises individuellement à deux courants parallèles qui stimulent le sens de l'olfaction et qui sont séparés par un fort gradient de concentration. Les crevettes ont détecté et fui le pentachlorophénol en concentration supérieure à 91 $\mu g \cdot L^{-1}$ dans l'eau de mer synthétique. Ce seuil de détection reflète les limites de l'étude statistique, et avec répétition de l'expérience, le seuil physiologique pourrait probablement être abaissé considérablement. Le pentachlorophénol semble agir davantage comme répulsif lorsqu'il est dissous dans l'eau estuarienne de la baie Galveston, Texas. La CL_{50} 96-h du pentachlorophénol a été évaluée à 317 $\mu g \cdot L^{-1}$; cela indique que les postlarves sont capables de fuir les concentrations fortement toxiques de ce pollutant. Chez les postlarves de cette espèce, le comportement d'évitement semble constituer un indicateur plus sensible de la réaction à des contaminants que des bioessais de toxicité du type classique.

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Galveston Bay is a highly industrialized estuary which receives over half of Texas' permitted wastewater discharges (Whitledge and Ray 1989). Acutely toxic pollutant effects are usually confined to the immediate vicinity of discharge points and seldom persist over widespread areas (Beitinger and Freeman 1983). For this reason, it is important to evaluate sublethal impacts. Many aquatic organisms can detect and avoid pollutants present in sublethal concentrations and this behavior is evident at almost all phylogenetic levels (Olla et al. 1980). Such avoidance is well documented for fishes (Hansen 1969; Folmar 1976; Lewis and Livingston 1977; Larrick et al. 1978; Maynard and Weber 1981; DeGraeve 1982; Gray 1990) and to a much lesser extent for crustaceans (Matthews 1969; Hansen et al. 1973; Kiørboe and Møhlenberg 1983). The presence of pollutants in repellent concentrations can potentially deny aquatic organisms access to large areas of otherwise suitable habitat. Some further evidence exists that low nonrepellent concentrations of pollutants can subvert adaptive responses such as attractions to food odors (Steele et al. 1985, 1987, 1990).

Pentachlorophenol and sodium pentachlorophenate are broad-spectrum biocides which are collectively the most heavily used biocides in the United States (Cirelli 1978). Worldwide annual production of pentachlorophenol exceeds 50 million kg, with U.S. and Canadian production accounting for approximately 22.7 million kg (Eisler 1989). Pentachlorophenol is a potent insecticide classified by the U.S. Environmental Protection Agency (EPA) as a priority pollutant. Its primary applications are as a wood preservative and termiticide with secondary employment as a fungicide, herbicide, algicide, disinfectant, and antifoulant (Cirelli 1978; Eisler 1989).

Studies conducted on grass shrimp (*Palaemonetes pugio*) and blue crab (*Callinectes sapidus*) suggest that pentachlorophenol uncouples oxidative phosphorylation and interferes with carbohydrate metabolism, lipid metabolism, ion transport, and possibly protein metabolism (Rao et al.

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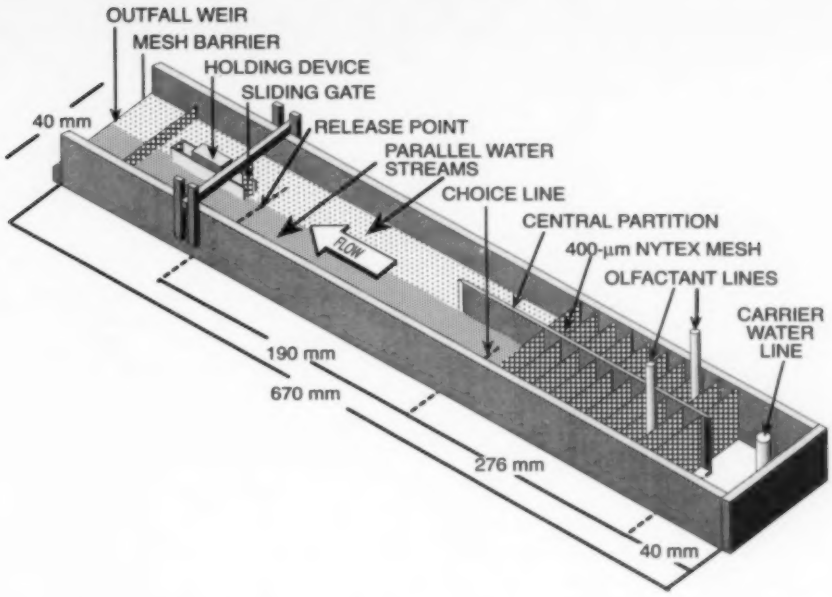

FIG. 1. Schematic diagram of the laminar-flow choice chamber indicating major dimensions. Diagram not to scale.

1979). Low concentrations of pentachlorophenol were associated with declines in species abundance and estuarine macrobenthos community diversity (Tagatz et al. 1977, 1983). In a study of chronic pentachlorophenol toxicity conducted in artificial streams, low concentrations were associated with reduced fish growth and reduced larval fish drift (Zischke et al. 1985). Pentachlorophenol has been detected in water, sediments, and a variety of estuarine organisms from West Galveston Bay, including juvenile brown shrimp (*Penaeus aztecus*) (Murray et al. 1981).

The brown shrimp is an estuarine-dependent species which supports a substantial commercial fishery in the Gulf of Mexico. Neither the acute toxicity nor behavioral avoidance of pentachlorophenol has been evaluated for postlarval penaeid shrimp. The objectives of this study were to measure avoidance of pentachlorophenol by postlarval *P. aztecus* using a recently developed laminar-flow choice chamber (Benfield and Aldrich 1991), to compare threshold avoidance and acutely toxic concentrations, and to determine whether low concentrations of pentachlorophenol might interfere with previously documented attraction to estuarine water (Benfield and Aldrich 1992).

Methods

Laminar-Flow Choice Chamber

The choice chamber and its associated seawater delivery system has been described by Benfield (1991) and Benfield and Aldrich (1991). This system has successfully been employed to measure attraction to Galveston Bay water (Benfield and Aldrich 1992) and to test the attractiveness of olfactants derived from salt marsh habitat components (Benfield 1991). The chamber consists of a rectangular glass flume which provides single test animals with equal exposure to two parallel olfactant streams in a 5 mm·s^{-1} current (Fig. 1). Carrier water (25‰ synthetic sea salt dissolved in 18 MΩ deionized water and filtered throuth 5-μm polyester and activated carbon filter cartridges) enters at the upstream end and is split into two parallel streams by a central partition which physically divides only the upstream portion of the chamber. Olfactants are introduced into opposite sides of the partition where the olfactants mix with, and are diluted in, the carrier water. Mixing is completed by the time the streams pass through the Nytex screens and enter the testing region of the chamber. Laminar-flow induced by passage through Nytex screens results in a steep concentration gradient between the two olfactant streams which persists along the length of the chamber. Replicate measurements of simulated olfactant (fluorescein dye) concentrations along the length of the chamber in both the olfactant and carrier water streams did not differ significantly ($p < 0.05$) from the empirical estimates based upon stock dye concentrations and the delivery rates of olfactants and carrier water (Benfield and Aldrich 1991). Individual postlarvae are released from downstream holding device and permitted to move upstream for 190 mm until crossing an arbitrary choice line. The steep gradient between olfactant streams permits unrestricted movement across a stable olfactant interface. This system can measure responses to a single olfactant and a carrier water control or to a pair of olfactants. Each experiment consisted of approximately 50 individual trials which each evaluated the response of a single animal to a given pentachlorophenol concentration. By assigning the olfactant to the left or right side in each trial in a random sequence with equal overall frequency, it was possible to evaluate preference or avoidance with a χ^2 goodness of fit test. Independence of selection for chamber side (left or right) and the treatment (pentachlorophenol or control) was assessed with a χ^2 test of independence. The movement pattern of each animal was recorded manually by tracing its path on a tem-

plate of the chamber while timing the duration of each trial. Subsequent analyses of this data permitted turning direction and frequency to be calculated. Turns were defined as any change in direction >90°. Differences in the upstream and downstream turning frequencies were assessed with a Mann–Whitney *U* test.

Pentachlorophenol Assay

Pentachlorophenol concentrations were measured using the method of Carr et al. (1982). This spectrophotometric technique was developed to quantify pentachlorophenol in seawater samples and has detection limits of approximately 30 $\mu g \cdot L^{-1}$ for 500-mL samples. In the absence of a 320-nm-capable spectrophotometer, we modified the technique by using 325 nm after confirming that the substituted wavelength was near the apex of a broad absorbance peak.

Pentachlorophenol standards were prepared over the 0–500 $\mu g \cdot L^{-1}$ range by dissolving analytical-grade pentachlorophenol (Sigma Chemical) in 0.3 mL of reagent-grade acetone (99.5%) and making up tthe volume to 500 mL with 25‰ synthetic seawater. Acetone served as a carrier solvent which facilitated the rapid dissolution of pentachlorophenol in seawater. Absorbances of extracted seawater and 500 mL of seawater plus 0.3 mL of acetone were similar, which suggested that the acetone did not interfere with the assay.

All glassware used in the pentachlorophenol analyses was washed with detergent (Alconox or Liquinox), rinsed in tapwater, and then rinsed sequentially with 1.2 M HCl, deionized water, acetone, and 18 MΩ deionized water. After each experiment the choice chamber was soaked in detergent for at least 4 h and then flushed thoroughly with tap water followed by rinses with 1.2 M HCl and 18 MΩ deionized water.

Indirect Estimation of Exposure Pentachlorophenol Concentrations

Stock solutions of pentachlorophenol were pumped into the choice chamber where they mixed with, and were diluted in, the synthetic carrier water. The 500-mL sample volume required for the pentachlorophenol analysis precluded direct collection from within the choice chamber. For this reason, exposure concentrations were estimated indirectly by first measuring the stock pentachlorophenol concentration and then estimating the stock solution to carrier water dilution ratio with fluorescein dye.

Stock solutions were prepared by adding analytical-grade pentachlorophenol dissolved in 2 mL of acetone to 13.5 L of carrier water (25‰ carbon-filtered synthetic seawater prepared from Fritz Supersalt dissolved in 18 MΩ deionized water) in a glass carboy. The stock solution was pumped into one randomly selected side of the choice chamber, while carrier water was dispensed into the opposite side at the same rate. Between individual trials, the feed line was directed into a 500-mL volumetric flask while the previous test animal was removed and the chamber was flushed with carrier water and refilled. Using this method, it was possible to accumulate a sufficiently large (500 mL) pentachlorophenol sample for analysis after approximately 12 trials. Samples were analyzed as soon as 500 mL had been collected. The concentrations of three such outlet samples were averaged to estimate the mean stock pentachlorophenol concentration. After the chamber was refilled the feed lines were reconnected and the olfactant streams were permitted to reestablish and stabilize over a 4-min period.

Estimation of fluorescein dye concentations in the chamber required only 1–2 mL which could be withdrawn with negligible disturbance of the laminar flow. This provided a convenient technique for estimating the stock solution to carrier water dilution ratio. Prior to the first trial, and at intervals of 12 and 24 trials, fluorescein dye was dissolved in 250-mL aliquots of the test solution and pumped into one side of the chamber at the same flow settings used for the pentachlorophenol. Carrier water was dispensed into the opposite site at the same rate. Samples of dye were withdrawn by pipette from the middle of the dye stream at a point in line with the choice line. The feed lines were reversed and a second dye sample was collected from the opposite side of the chamber. Sample absorbances were measured in a spectrophotometer at 480 nm using a carrier water blank and converted to concentrations with a standard curve. The concentrations of each pair of dye samples were averaged and divided by the stock dye concentration to estimate dilution in the chamber. Each dilution estimate was then multiplied by the mean stock pentachlorophenol concentration. This provided three point estimates of the pentachlorophenol concentration in the chamber which were averaged to estimate the mean exposure concentration during each experiment. Dye flow was also observed at 12 trial intervals throughout each experiment to confirm the stable separation of the olfactant and carrier water streams.

Pentachlorophenol Avoidance Experiments

Postlarvae were collected from the surf zone of Galveston Island with a 1-m beam trawl and transported to the laboratory in 20-L buckets of seawater where *P. aztecus* were separated from other planktonic organisms. Shrimp were initially held in aerated 500 mL beakers which contained 200-μm-filtered beach water until the temperature had equilibrated to within 3°C of the laboratory temperature (approximately 25°C). The salinity of the water was then adjusted to 25‰ in steps of approximately 3‰ using deionized water or synthetic sea salts after which postlarvae were transferred to a 60-L aquarium containing 25‰ synthetic carrier water. Each aquarium was equipped with a power canister filter containing activated carbon. Postlarvae were held under constant overhead fluorescent illumination and fed daily with small amounts of finely ground flake food (Tetra-Marin). Postlarvae were tested between 1 and 7 d of captivity.

In the absence of published pentachlorophenol LC_{50} values for postlarval *P. aztecus*, the acute criterion for marine water of 53 $\mu g \cdot L^{-1}$ (United States Environmental Protection Agency 1980) formed the basis for the initial exposure concentration. Beginning at this concentration, tests were conducted at progressively higher concentrations using approximately 50 $\mu g \cdot L^{-1}$ increments up to 400 $\mu g \cdot L^{-1}$ with two additional experiments at 0 $\mu g \cdot L^{-1}$. These latter experiments contained acetone (0.002 M) without pentachlorophenol which permitted an evaluation of postlarval responses to the dilute acetone present in all studies. At least two experiments were conducted at all concentrations except 50 $\mu g \cdot L^{-1}$ which was evaluated once. Experiments were conducted between May and July 1991. Because we were concerned that short-term pentachlorophenol exposure might impair postlarval ability to make a choice, the maxi-

mum time to leave the chamber and make a choice was restricted to 20 min. Trials in which animals failed to make a choice after 20 min were repeated using a new animal. All other experimental protocols were identical to those described for estuarine water in Benfield and Aldrich (1992). Each experiment was designed to evaluate the responses of 50 *P. aztecus* postlarvae. Shortages of postlarvae reduced the sample sizes in one 0 $\mu g \cdot L^{-1}$ (n = 38) and the 50 $\mu g \cdot L^{-1}$ experiment (n =35).

The percentage of animals which avoided the pentachlorophenol (selected the control stream) was plotted against the mean exposure concentration in each experiment. The relationship between these variables was quantified with a least squares regression which included 90% confidences on the mean. The avoidance threshold was interpolated from the intercept of 50% avoidance and the lower boundary of the confidence interval of the mean avoidance predicted by the regression. Since only 5% of all means would be expected to fall below the lower confidence interval, the remaining 95% should fall above the lower confidence interval. On this basis, our criterion reflects the concentration which would generate mean avoidances of 50% or more in 95% of all experiments.

Avoidance of Pentachlorophenol in Estuarine Water

Our earlier studies had demonstrated a significant preference by postlarval brown shrimp and white shrimp (*Penaeus setiferus*) for estuarine water over carbon-filtered carrier water (Benfield and Aldrich 1992). Three experiments were conducted at pentachlorophenol concentrations similar to the marine chronic criterion of 34 $\mu g \cdot L^{-1}$ (United States Environmental Protection Agency 1980) to determine whether low levels of pentachlorophenol reduced the attractiveness of estuarine water. The current EPA criterion has been reduced to 7.9 $\mu g \cdot L^{-1}$ (United States Environmental Protection Agency 1986). Water was collected in August 1991 from a west Galveston Bay salt marsh at the same point as the estuarine water samples used in our earlier studies (Benfield and Aldrich 1992). Samples were passed through a 5-μm polyester filter and returned to the laboratory in Nalgene containers where samples were diluted to the carrier water salinity (25‰) with 18 MΩ deionized water. Stock solutions were prepared by adding pentachlorophenol dissolved in 2 mL of acetone to 13.5 L of estuarine water. Postlarvae were given a choice of estuarine water and a solution of pentachlorophenol in estuarine water. The estuarine water contained an unknown compound which absorbed at 325 nm but differed from the absorbance curve of pentachlorophenol. Because this compound inflated the pentachlorophenol concentration estimates, absorbances of three replicate estuarine water samples were averaged and subtracted from the pentachlorophenol/estuarine water sample absorbances to correct for this problem.

Acute Toxicity Bioassay

The acute toxicity of pentachlorophenol to postlarval brown shrimp was estimated with a 96-h LC_{50}. The methodology was based upon EPA protocols established for the mysid *Mysidopsis bahia* (Peltier and Weber 1985). A preliminary screening experiment suggested that the LC_{50} was below 400 $\mu g \cdot L^{-1}$. The LC_{50} was estimated in duplicate experiments conducted during August 1991 using 100, 200, 300, 400, and 500 $\mu g \cdot L^{-1}$ concentrations of pentachlorophenol. Stock toxicants were prepared by dissolving reagent-grade pentachlorophenol in 300 μL of acetone which was added to 2 L of 25‰ carrier water. The experiment included both synthetic seawater and acetone (2 L of synthetic water + 300 μL of acetone) controls. Tests were conducted in 250-mL Erlenmeyer flasks which received gentle aeration via glass Pasteur pipettes.

Postlarvae were collected from the Galveston beachfront 2 d prior to each experiment and held at 25‰ under the same conditions described for the avoidance experiments. Treatments and controls were randomly assigned to duplicate flasks and 10 postlarvae were placed in each flask in a random sequence. Residual holding water was then removed from each flask with a pipette and replaced with 200 mL of the appropriate test solution. A pair of overhead 40-W fluorescent tubes provided illumination with a 12 h light : 12 h dark photoperiod, while a thermostat maintained room temperature at approximately 25°C. Test solutions were replaced with 200 mL of the stock toxicant every 24 h. Two 500-mL aliquots from each treatment were analyzed for pentachlorophenol and averaged to estimate the mean exposure concentration. Approximately 2 h prior to solution replacement, the animals were fed a small amount of Tetra-Marin flake food. Immediately prior to replacing the solutions, we measured the dissolved oxygen and temperature of each flask. The old toxicant, dead animals, and uneaten food were removed with a pipette and the surviving postlarvae were counted. After 96 h the animals surviving in each flask were counted and a random sample of 10 postlarvae was preserved for measurement. Mean total lengths and wet masses of postlarvae with 95% confidences were 10.0 ± 0.4 mm and 0.0021 ± 0.0002 g (experiment 1) and 10.0 ± 0.5 mm and 0.0024 ± 0.0003 g (experiment 2).

The LC_{50} was estimated for each experiment using the probit technique described in Peltier and Weber (1985). Percent mortalities in each flask were converted to probits and plotted as a function of the logarithm of the mean exposure concentration. The LC_{50} for each experiment was interpolated from a least squares regression of these variables and averaged to estimate the mean acute toxicity.

Results

Avoidance of Pentachlorophenol in Synthetic Seawater

There was a significant linear relationship (F = 15.253, p = 0.0013) between postlarval brown shrimp avoidance and the mean pentachlorophenol concentration (Fig. 2). Based on this regression, the threshold pentachlorophenol concentration which would be expected to elicit avoidances above 50% in 95% of all trials was approximately 91 $\mu g \cdot L^{-1}$.

Considerable variation in the degree of avoidance was evident among experiments and responses for five experiments fell well outside the 90% confidence limits of the regression. There were no obvious reasons for these outlying observations although variability inherent in different batches of postlarvae from several cohorts may have contributed. Comparisons of time in captivity, mean overall choice times, and choice times for pentachlorophenol and the control failed to indicate any common factor which might explain this variability.

Postlarvae did not appear to avoid the odor of acetone in the carrier water and although postlarvae selected the stream containing acetone with marginally greater frequency, these

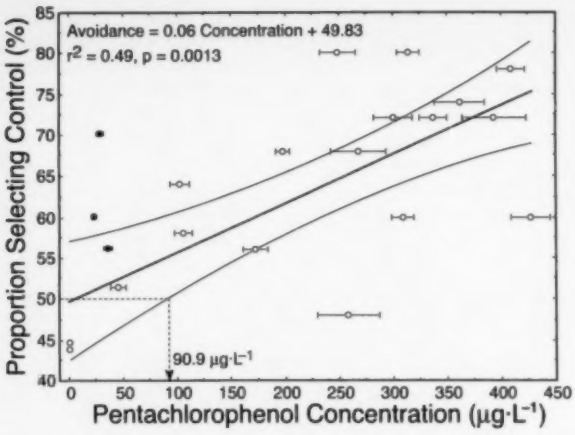
Avoidance = 0.06 Concentration + 49.83
r2 = 0.49, p = 0.0013
Proportion Selecting Control (%)
Pentachlorophenol Concentration (µg·L-1)
90.9 µg·L-1

FIG. 2. Relationship between avoidance and pentachlorophenol concentration in synthetic seawater (○) and estuarine water (●). Upper and lower bounds reflect 90% confidence invervals on the mean predicted avoidance based upon results obtained in synthetic seawater. Horizontal bars indicate 95% confidences on mean pentachlorophenol concentrations. The minimum pentachlorophenol concentration which would be expected to evoke avoidance ($p \leq 0.05$) is 90.9 $\mu g \cdot L^{-1}$.

differences were not significant. The selection frequency predicted for the 0.002 M acetone control (0 µg pentachlorophenol·L^{-1}) by the regression equation was 49.83% (Fig. 2).

Selections for chamber side and the treatment were independent in all experiments. Significant preference for one side of the chamber ($\chi^2 = 5.12$, $p = 0.024$) was evident in one experiment (379.68 $\mu g \cdot L^{-1}$). The cause of this bias could not be determined and the experiment was excluded from the analysis by an a priori decision.

The mean time to make a choice was 59.1 s and choice time did not differ significantly among experiments ($F = 1.04$, $p = 0.406$). The mean choice times of trials which selected the pentachlorophenol solution (53.5 s) or the control (60.8 s) were not significantly different among experiments ($F = 1.00$, $p = 0.469$). No significant relationships were apparent between pentachlorophenol concentration and the mean choice time ($F = 0.419$, $p = 0.527$), choice time for trials in which postlarvae selected the test solution ($F = 2.115$, $p = 0.165$), or choice times for trials in which postlarvae selected the control ($F = 0.015$, $p = 0.906$). The number of trials which had to be repeated because animals failed to leave the chamber or make a choice in 20 min ranged from 0 to 6 (mean 1.6) in each experiment.

In the 16 experiments containing detectable pentachlorophenol, postlarvae were more likely to turn downstream in the pentachlorophenol stream and upstream in the control stream (Table 1). The mean upstream and downstream turning frequencies were significantly different ($Z = -2.678$, $p = 0.0074$) in the carrier water stream only, with the opposite nonsignificant trend evident in the pentachlorophenol ($Z = -1.873$, $p = 0.0611$).

Avoidance of Pentachlorophenol in Estuarine Water

Pentachlorophenol concentrations well below the detection threshold were not expected to evoke avoidance behavior in postlarvae. Accordingly, estuarine water containing a low concentration of pentachlorophenol and unadulterated estuarine water should have been equally attractive to test animals. Given such a choice, the postlarvae chose the estuarine water without pentachlorophenol with consistently greater frequency in each experiment (Table 2). Significant avoidance ($p < 0.05$) of the estuarine water containing pentachlorophenol was evident at an intermediate concentration (28.45 $\mu g \cdot L^{-1}$) but not at the highest concentration tested.

The percent avoidance in all three experiments fell above the lower 90% confidence limit of the avoidance–concentration regression (Fig. 2) and all produced avoidances above 50% at concentrations well below the 90.9 $\mu g \cdot L^{-1}$ threshold. This suggests that the attractiveness of estuarine water was diminished by pentachlorophenol concentrations which were not repellent in synthetic seawater.

The turning pattern in the pentachlorophenol + estuarine water experiments was consistent with that observed in higher concentrations of pentachlorophenol dissolved in synthetic seawater. These differences were not significant among the three experiments in either the estuarine water + pentachlorophenol stream ($Z = -0.66$, $p = 0.5019$) or the estuarine water stream ($Z = -0.929$, $p = 0.3527$).

TABLE 1. Comparisons of the mean upstream and downstream turning frequencies in the pentachlorophenol and synthetic seawater streams. Turns defined by >90° change in direction. Tests based on 32 trials which included at least one downstream and upstream turn. Probabilities indicate outcomes of Mann–Whitney *U* tests comparing upstream and downstream turning frequencies in each water type.

	Mean turning frequency (±SE)	
Water type	Upstream	Downstream
	$p = 0.061$	
Pentachlorophenol	0.679 ± 0.075	0.926 ± 0.097
	$p = 0.007$	
Carrier water	0.797 ± 0.061	0.549 ± 0.078

Acute Toxicity of Pentachlorophenol

The two acute toxicity experiments yielded LC_{50} estimates of 269 and 365 µg pentachlorophenol·L^{-1} (means = 316.9 $\mu g \cdot L^{-1}$) (Fig. 3). The physical conditions in each flask appeared adequate for postlarvae, and survival in both the seawater and 0.002 M acetone controls ranged from 80 to 90% with identical means of 85%. Dissolved oxygen concentrations and flask temperatures were similar in both experiments. Mean dissolved oxygen concentration ranges (±95% confidence limits) were 6.23 ± 0.06 to 6.34 ± 0.05 and 5.72 ± 0.15 to 6.42 ± 0.08 $mg \cdot L^{-1}$ in experiments 1 and 2, respectively. Corresponding mean temperature ranges (±95% confidence limits) were 24.7 ± 0.1 to 25.3 ± 0.1 and 25.2 ± 0.1 to 25.4 ± 0.1°C.

Discussion

Avoidance bioassays are frequently used to evaluate sublethal effects of pollutants. Most studies have focused on fishes, and responses have been reviewed by Brown et al. (1982) and Beitinger and Freeman (1983). The linear relationship between avoidance and exposure concentration

TABLE 2. Relative selection of estuarine water and pentachlorophenol in estuarine water by postlarval *P. aztecus* at three pentachlorophenol concentrations (mean exposure concentrations). Probabilities indicate significance levels for χ^2 tests of independence, χ^2 goodness of fit tests for left/right chamber bias, and χ^2 goodness of fit tests for avoidance of pentachlorophenol in estuarine water.

Pentachlorophenol concentration ($\mu g \cdot L^{-1}$)	Olfactant stream selected		Probability		
	Estuarine water	Estuarine water with pentachlorophenol	Independence	Chamber bias	Avoidance
22.99	30	20	0.773	1.000	0.157
28.45	35	15	1.000	1.000	0.005
35.06	28	22	0.828	0.572	0.396

observed in this study was also evident in evaluations of detection thresholds of pinfish (*Lagodon rhomboides*) and gulf killifish (*Fundulus grandis*) for bleached kraftmill effluent (Lewis and Livingston 1977), coho salmon (*Oncorhynchus kisutch*) for monocyclic aromatics (Maynard and Weber 1981), and brook trout (*Salvelinus fontinalis*) for low pH (Pedder and Maly 1986).

This study appears to be the first evaluation of pollutant avoidance by any penaeid. The avoidance threshold of 90.9 μg pentachlorophenol$\cdot L^{-1}$ is a conservative estimate which reflects limitations imposed by statistical power. The linear relationships obtained in the previously cited avoidance studies and present research suggest that animals are capable of detecting and avoiding sublethal toxicant concentrations. With additional replication or use of chamber designs permitting greater statistical power, it is theoretically possible to detect avoidance at much lower concentrations. We recognize that detection of the olfactory stimulus may not evoke avoidance behavior which can be detected within the confines of our choice chamber. Other choice systems or different measures of olfactory stimulation such as antennular flicking rates (Olla et al. 1980; Rebach et al. 1990) or neurophysiological studies may be required to determine the absolute detection threshold.

Few pentachlorophenol avoidance data are available for fish. Pentachlorophenol was repellent to green sunfish (*Lepomis cyanellus*) between 5000 and 20 000 $\mu g \cdot L^{-1}$ (Summerfelt and Lewis 1967) and these authors cited another study which suggests that fish can detect pentachlorophenol above 10 000 $\mu g \cdot L^{-1}$. The lack of avoidance data and poor control over exposure concentrations in Summerfelt and Lewis' study made this result difficult to interpret. Their observation that fish showed signs of distress at both 5000 and 20 000 $\mu g \cdot L^{-1}$ suggests that they overestimated the avoidance threshold. None of our test animals showed obvious signs of distress within the choice chamber up to the maximum pentachlorophenol concentration.

In the present experiments with shrimp postlarvae, the generally consistent patterns of upstream and downstream turning suggest that postlarvae turn and move upstream upon encountering an attractive odor or leaving a repellent odor. This pattern is reversed when encountering a repellent odor or leaving an attractive odor. In an earlier study of postlarval responses to estuarine water (Benfield and Aldrich 1992), both *P. aztecus* and *P. setiferus* postlarvae tended to move upstream when entering and downstream when leaving an attractive stimulus (estuarine water). Attractive and repellent odors may release positive and negative rheotaxic responses. Movement frequently carried postlarvae along

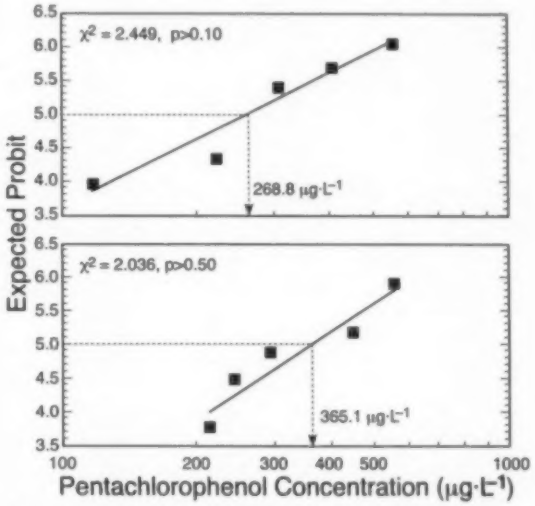

FIG. 3. Acute toxicity of pentachlorophenol to postlarval *P. aztecus* in two replicate 96-h experiments. LC_{50} interpolated from intersection of the 5.0 probit which corresponds to 50% mortality. χ^2 indicates fit of regression (a nonsignificant χ^2 implies that the data are adequately represented by the regression line).

the boundary of the two streams, suggesting a tropotaxic component to orientation. Tropotaxes are adaptive in very steep gradients (Larrick et al. 1978). While the steep concentration gradient used in this study may be considered artificial, there are phenomena in estuaries such as discharge plumes, downstream eddies of plumes, freshwater outflows, and haloclines which are bounded by steep concentration gradients. Unfortunately the selection times could not be used to evaluate orthokinetic responses because instantaneous shrimp velocities in the two odor streams could not be determined from visual records. A larger behavioral space and video recording of movement would improve our understanding of how these animals orient towards odor.

The artificiality of the synthetic seawater used in our system was a necessary compromise which satisfied the physiological requirements of the postlarvae without introducing the chemical and olfactory variability inherent in estuarine waters such as Galveston Bay. The influence of terrestrial runoff extends a long distance off the upper Texas coast and a supply of more consistent oceanic water was not readily available. Future tests conducted with diluted offshore water as a carrier would further improve the system.

Avoidance is usually one of the first responses of an organism to an environmental stress imposed by a toxicant (Beitinger and Freeman 1983). Avoidance behavior is the primary mechanism for ensuring that animals can evade deleterious or undesirable conditions (Larrick et al. 1978). The principal utility of acute toxicity bioassays is to predict toxicity of compounds to species rather than environmental hazard (Hansen 1984). Pollutants seldom attain acutely lethal concentrations except in the immediate vicinity of discharge points (Beintinger and Freeman 1983). The presence of toxins at sublethal repellent concentrations may deny large areas of suitable habitat to organisms. Avoidance bioassays generally detect responses at concentrations below lethality (Summerfelt and Lewis 1967; Folmar 1976) and for this reason can have more practical utility than toxicity bioassays provided that the toxicant can be detected by the test animal. The avoidance studies in synthetic seawater suggest that postlarval penaeid shrimp are capable of avoiding pentachlorophenol at concentrations well below the 96-h LC_{50}. The avoidance regression and the responses to pentachlorophenol in estuarine water further suggest that detection and avoidance of pentachlorophenol may occur at less than 10% of the LC_{50}.

Although there do not appear to be any published estimates of the 96-h pentachlorophenol LC_{50} for postlarval penaeids, the LC_{50} for juvenile pink shrimp (*Penaeus duorarum*) was estimated to be 5600 $\mu g \cdot L^{-1}$ (United States Environmental Protection Agency 1980). Larval stages are generally more sensitive to toxicants than juveniles or adults. Our results are comparable with estimates for other small crustaceans. The 96-h LC_{50} for *M. bahia* was 320 $\mu g \cdot L^{-1}$ (Mayer 1987), while estimates for *Palaemonetes pugio* ranged from 400 to 1200 $\mu g \cdot L^{-1}$ (Eisler 1989). The variability in toxicity is probably due in part to molt stage of the test animals, as Conklin and Rao (1978) found that grass shrimp were more sensitive to sodium pentachlorophenate following ecdysis. The present results also compare favorably with 223–392 $\mu g \cdot L^{-1}$ 96-h LC_{50} estimates for 2- to 6-wk fry of sheepshead minnow (*Cyprinodon variegatus*) (Borthwick and Schimmel 1978).

The variability in our estimates of the LC_{50} is comparable with variation obtained by other laboratories. An EPA multilaboratory comparison of the precision of replicate LC_{50} estimates from 92 reference toxicant tests conducted on three species had a mean coefficient of variation (CV) of 38% and a range of 22–187% (Peltier and Weber 1985). The same study indicated that a CV of 40% was obtained from 12 replicate 96-h LC_{50} experiments using sodium pentachlorophenate and fathead minnow (*Pimephales promelas*). The CV from our study (21.48%) appears acceptable in view of such variation.

The pentachlorophenol concentrations used in the estuarine water were near the 1980 EPA marine chronic water criterion of 34 $\mu g \cdot L^{-1}$ (United States Environmental Protection Agency 1980). This criterion has subsequently been reduced to 7.9 $\mu g \cdot L^{-1}$ (United States Environmental Protection Agency 1986) and Eisler (1989) has recommended that the maximum allowable aquatic criterion be reduced to 3.2 $\mu g \cdot L^{-1}$. While these concentrations fall well below the 90.9 $\mu g \cdot L^{-1}$ avoidance threshold obtained in our study, the enhanced avoidance noted in estuarine water containing low concentrations of pentachlorophenol suggests a need for further reseach.

In a previous study using this choice chamber, we demonstrated that water from a Galveston Bay saltmarsh was attractive to postlarval *P. aztecus* and *P. setiferus* (Benfield and Aldrich 1992). Odors may provide immigrating postlarvae with both orientative and habitat quality information. The responses of postlarvae to pentachlorophenol in estuarine water suggest that the presence of low concentrations of this pollutant may reduce the attractiveness of Galveston Bay water. If odor assists postlarvae in locating estuaries or nursery habitats, then sublethal concentrations of pentachlorophenol or other compounds may impede normal orientation.

Subversion of adaptive responses to odor by pollutants has been documented. Sublethal concentrations of copper (1 $\mu g\ Cu \cdot L^{-1}$) which were not repellent were capable of suppressing a positive feeding response by zebrafish (*Brachydanio rerio*) to 10^{-3} M alanine (Steele et al. 1987, 1990). Similar abolishment of attraction was noted in longnose killifish (*Fundulus similis*) and diamond killifish (*Adinia xenica*) (Steele et al. 1985). Olla et al. (1980) suggested that pollutants may decrease the signal-to-noise ratio of natural chemical cues to the point where they can no longer be sensed. The variability we noted in three experiments suggests the need for further study of the interaction between pollutants and attraction of postlarvae to estuarine water. Enhanced avoidance may also have been a consequence of chemical interaction(s) between pentachlorophenol and organic compounds in the estuarine water which produced a repellent compound or a supraadditive response to pentachlorophenol and another undetected pollutant. Galveston Bay is a highly industrialized system which receives discharges of a wide range of pollutants. It is possible that some of the interannual variation in year class success may be attributed to differential attractiveness of the bay water. Most commercially important fish and crustaceans which use Galveston Bay are catadromous migrants which enter the estuary at early life stages. Behavioral avoidance represents a potentially useful direction for evaluating the responses of penaeid shrimp, and possibly other species, to a variety of relevant toxicants.

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Survival, Distribution, and Ion Composition in Two Strains of Brook Trout (*Salvelinus fontinalis*) Fry after Exposure to Episodic pH Depressions in an Adirondack Lake

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Van Offelen, H.K., C.C. Krueger, C.L. Schofield, and C. Keleher. 1994. Survival, distribution, and ion composition in two strains of brook trout (*Salvelinus fontinalis*) fry after exposure to episodic pH depressions in an Adirondack lake. Can. J. Fish. Aquat. Sci. 51: 792–799.

Brook trout (*Salvelinus fontinalis*) fry are susceptible to high mortality in acidified waters because of their low tolerance to acidic conditions and the potential occurrence of this life stage with episodic acidification associated with snowmelt. Prior to snowmelt, equal numbers of Assinica and Temiscamie strain fry were placed into enclosures. Twelve days after stocking, no consistent differences between strains were observed in survival, distribution, or ion composition. No fry of either strain survived in enclosures placed in shallow water (pH 4.8; 0.7 m depth). Fifty to 100% of fry of both strains survived in enclosures placed in deep water (pH 6.3; >2.0 m depth). Fry survival in long enclosures that extended from shallow to deep water was 80–99%. Within long enclosures, both strains were recovered in higher densities from middle and deep sections of the enclosures than from shallow sections. High fry survival and nonrandom distributions in the long enclosures indicated that both strains were able to avoid lethal, nearshore waters during spring snowmelt by moving to deeper water.

La mortalité peut être élevée lorsque les alevins de l'omble de fontaine (*Salvelinus fontinalis*) se trouvent en eaux acides car ils tolèrent mal l'acidité. Or, il est possible que ce stade de vie coïncide avec l'acidification épisodique des plans d'eau associée à la fonte des neiges. Avant celle-ci, des alevins des souches Assinica et Temiscamie ont été introduits en nombre égal dans des parcs. Douze jours après l'ensemencement, il n'y avait pas de différence établie entre les souches sur les plans de la survie, de la distribution ou de la composition ionique. Peu importe les souches, aucun alevin n'a survécu dans les parcs placés en eau peu profonde (pH 4,8; 0,7 m de profondeur). Cinquante à 100 % des alevins des deux souches ont survécu dans les parcs placés en eau profonde (pH 6,3; plus de 2,0 m de profondeur). La survie des alevins dans les longs parcs qui s'étiraient des eaux peu profondes aux eaux profondes, a été de 80 à 99 %. Dans ce dernier cas, les alevins des deux souches ont été récupérés en plus grande densité dans la section centrale et la section profonde des parcs, plutôt que dans la section peu profonde. Le taux élevé de survie des alevins et les distributions non aléatoires dans les grands parcs indiquent que les deux souches sont capables d'éviter les eaux peu profondes et létales durant la fonte des neiges en allant en profondeur.

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Acidification of lakes and streams has had adverse effects on fish populations throughout northeastern North America (Haines 1981; Schofield 1982; Kelso et al. 1986). Changes in fish population structure and abundance, indicative of recruitment failure, have been observed in these acidified lakes (Beamish and Harvey 1972; Harvey 1982; Frenette and Dodson 1984; Schofield and Driscoll 1987) and in whole-lake acidification experiments (Mills et al. 1987).

Extensive laboratory investigations with brook trout (*Salvelinus fontinalis*) have demonstrated that exposure of fertilized eggs, alevins, and fry to natural acidic conditions (low pH, high total Al, low Ca) can result in high mortality. For example, brook trout emergent fry suffered mortalities of 94, 82, and 100% when exposed to waters with pH 4.2, 4.6, and 5.2 and elevated Al levels, respectively (Baker and Schofield 1982). Fertilized eggs and embryos exposed to similar conditions suffered significantly less mortality than alevins and fry. Lethality of acidic conditions to brook trout has been shown to depend not only on pH and the life stage exposed but also on the concentrations of Al and Ca present in the water (e.g., Wood et al. 1988). Sensitivity to pH typically decreases with age whereas sensitivity to Al exposure increases with age, while increasing Ca concentrations tend to reduce sensitivity of all life stages to acid and Al (e.g., Ingersoll et al. 1990b).

In addition to being physiologically sensitive to acidic conditions, early life stages of brook trout occur simultaneously with acidification associated with snowmelt. Runoff from melted snow may inundate nearshore areas of lakes with water of lethal low pH and high Al concentrations (Cronan and Schofield 1979; Jeffries et al. 1979; Gunn and Keller 1984; Galloway et al. 1987). Brook trout spawn in the fall often in groundwater seepage areas where pH levels may be higher than those of surface waters (Webster and

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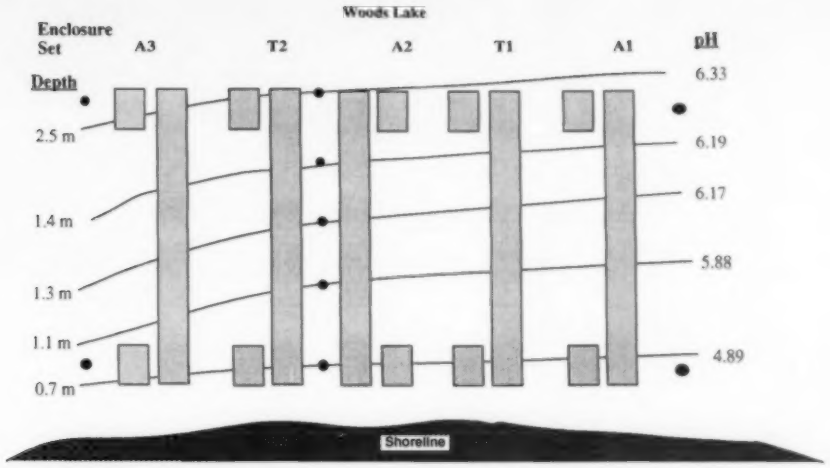

FIG. 1. Position of horizontal enclosures on the bottom of Woods Lake. Three sets of enclosures (each set is one long and two short enclosures) were stocked with Assinica strain (A1–A3) brook trout and two sets were stocked with Temiscamie strain (T1 and T2) brook trout. Depths and pH levels represent conditions at the end of the study. Circles indicate the locations where water was sampled.

Eiriksdottir 1976; Johnson and Webster 1977). Eggs located in these seepage areas would probably be protected if surface waters became acidic; however, alevin development and fry emergence from substrates often coincide with snowmelt events. In turn, mortality of young brook trout in acidified waters may be expected to depend on the intensity and duration of episodic acidification, the quantity and quality of groundwater seepage in the spawning redds, the mixing of acidic surface water with deeper layers, and the behavioral response of young fish to acidified water (see Gunn 1986).

Laboratory investigations have provided evidence that brook trout alevins and juveniles actively avoid low-pH waters, particularly when the water contains high concentrations of Al. Brook trout alevins were observed to actively avoid pH less than 5.0 and this response increased when test waters contained Al (33–500 μg/L; Gunn and Noakes 1986). In another study, brook trout juveniles similarly avoided test waters with pH <5.5 (Pedder and Maly 1986). Other sublethal responses to acidified water include changes in whole-body ion composition. Whole-body ions (Ca, Na, K) are expected to decline after sublethal exposure to acidic conditions (low pH, elevated Al; Wood et al. 1990). Sublethal reductions in these ions have contributed to reduced fitness in lake trout alevins exposed to acidic conditions (Gunn and Noakes 1987).

Direct extrapolation of these laboratory observations to natural systems may not be valid because changes that occur in nearshore areas during episodic pH depressions are difficult to simulate. During natural pH depressions, lake level, ice thickness, and light penetration, water temperature, dissolved oxygen, and other related chemical conditions stochastically change and interact. These variables are potential stimuli to early life stages of brook trout and may affect their net response to acidified nearshore waters. No field investigations have been available to compare laboratory results and to provide direct observations on the effects of episodic pH depressions associated with snowmelt on brook trout fry.

The objective of this study was to describe and compare the effects of natural episodic pH depressions on the survival, depth distribution, and whole-body ion content of two strains of brook trout fry in an Adirondack lake.

Study Site

This study was conducted in March 1990 over a 12-d period before ice-out in Woods Lake (43°52′N, 71°58′W), a 23-ha headwater lake located in the west-central region of the Adirondack Mountains, New York. The physical and hydrological characteristics of Woods Lake and its lake basin have been described by Staubitz and Zarriello (1989). The lake has been included in the Lake Acidification Mitigation Project (LAMP) since 1984 (see Porcella 1989). Circumneutral conditions have been maintained through applications of calcium carbonate directly to the lake in May 1985, September 1986, and October 1988. Catchments of the two perennial tributaries to Woods Lake were limed during October 1989 as part of the Experimental Watershed Liming Study (Porcella 1991). Despite these mitigative liming treatments, episodic pH depressions have been documented annually during spring snowmelt in shallow nearshore areas of the lake (Gubala and Driscoll 1991). Complete mortality of caged brook trout fry was observed during these episodes, in shallow (<1 m) water, where acidic meltwater depressed pH levels to 4.2–4.8. However, captive fry experienced little or no mortality at greater depth (2 m), where pH levels generally remained above 6.0 (LAMP, unpublished data).

The experiments in this study were conducted in the eastern nearshore zone of Woods Lake, which received intermittent tributary drainage from an unlimed area of the watershed. The substrate in the study area consisted of organic sediments greater than 0.5 m in depth.

Methods

Enclosure Construction and Placement

Five sets of three cylindrical enclosures were placed in Woods Lake in March 1990 before ice-out (Fig. 1). Long

TABLE 1. Mean dry weights of Assinica (A) and Temiscamie (T) strain brook trout fry from the hatchery and enclosures during the study ($N = 20$). Two standard errors of each estimate are given in parentheses. No live fry were recovered from the short–shallow enclosures. *Statistically different from fry at the start ($P < 0.05$).

Location	Date	Strain	Mean weight (mg)	% change from start
Hatchery	March 16, 1990	A	25.1 (3.2)	—
		T	16.7 (1.6)	—
Hatchery	March 27, 1990	A	20.3 (3.4)	−19
		T	24.5 (3.4)	48*
Short–deep enclosure	March 28, 1990	A	19.7 (2.7)	−22*
		T	16.3 (1.0)	−2
Long enclosure, deeper sections	March 28, 1990	A	24.8 (2.8)	−1
		T	15.0 (1.4)	−10
Long enclosure, shallow section	March 28, 1990	A	23.4 (2.4)	−7
		T	16.2 (1.6)	−3

enclosures (15.0 m in length) and short enclosures (2.0 m in length) were constructed of fiberglass mesh, sewn into cylinders approximately 25.0 cm in diameter.

Each set of enclosures was composed of one long enclosure and two short enclosures (Fig. 1). Long enclosures extended 15 m perpendicular from shore from a depth of approximately 0.7 m to approximately 2.5 m. Short enclosures were placed on the lake bottom parallel to the long enclosures. One short enclosure in each set extended from the shallow end of the long enclosure offshore (hereafter referred to as the short–shallow enclosure). The other short enclosure in each set extended from the deep end of the long enclosure inshore (hereafter referred to as the short–deep enclosure). Two short enclosures were also suspended vertically from the ice surface near the shallowest end of the enclosure sets. These short vertical enclosures permitted periodic assessments of mortality during the 12-d study.

Enclosures were placed in the lake 1 d prior to stocking. To place enclosures on the lake bottom, nearshore and offshore rectangular holes were cut into the ice. Five polypropylene lines were run under the ice between the holes. The enclosures were then placed on the ice surface near the offshore hole. Concrete-filled 3.81-cm plastic (PVC) pipes were then fitted through loops sewn in the ends of the enclosures to provide a secure fastening point for the lines. The lines under the ice were then connected to each set of enclosures and the enclosures were slowly pulled into the lake. After placement, the ends of enclosures were aligned in the nearshore hole and anchored with concrete blocks. Concrete blocks were also connected to the lines at the deep end. Lines to the surface remained attached to the anchors to permit stocking and removal. Prior to stocking, divers inspected the enclosures to ensure they were properly positioned on the lake bottom.

Enclosure Stocking

Assinica and Temiscamie strain brook trout were stocked into the enclosures. These strains originated from two adjacent river systems in northern Quebec (see Flick 1977; Cone and Krueger 1988; Van Offelen et al. 1993) and have been reported to be genetically different (Perkins et al. 1993). Fry were reared at the Brandon Park Hatchery in the northern Adirondacks in separate circular fiberglass tanks. Gametes for both strains were collected from brood stocks maintained in natural ponds in the Adirondack region. Fertilized eggs were incubated in hatchery water (pH > 7.0, Ca > 9.5 mg/L). At stocking, the Assinica strain fry ranged in age from approximately 71 to 81 d post-hatch, while the Temiscamie strain fry were from 58 to 68 d old. Exogenous feeding by Assinica and Temiscamie fry had begun 28 and 17 d before stocking, respectively. Because of their different stage of development, Assinica fry either did not change weight or lost weight during the experiment, while Temiscamie fry either did not change weight or gained weight (Table 1). Fry were not fed 24 h before stocking. Prior to stocking, a sample of 20 fish of each strain was collected from rearing tanks for whole-body ion analysis. Fry destined for each enclosure were counted and placed in oxygenated plastic bags for transport to the study site.

One day after the enclosures were placed into the lake (March 16), fry were stocked at a density of 20 fish/m enclosure length (40 fish per short enclosure, 300 fish per long enclosure). Three sets of enclosures were stocked with Assinica strain fry. Two sets were stocked with Temiscamie strain fry. The fish were stocked into the nearshore ends of both the long and short–shallow enclosures. The short–deep enclosures were stocked at their offshore end. Two short enclosures placed vertically under the ice to monitor mortality were stocked separately with 20 Assinica and 20 Temiscamie fry.

Enclosure Removal

Eleven days after stocking (March 27), divers working from the deep ends to the shallow ends divided each long enclosure into three sections by tying them off. The first section was the first 1–3 m meters of each long enclosure, from its deep end (approximately 2.5 m) to where the water was approximately 1.4 m deep. The second section extended from the end of the first section for 6–10 m to a depth of approximately 1.1 m, the shallowest point under the ice that a diver could reach. The third section was the remaining 4–8 m of the enclosure to the shallow end (approximately 0.7 m deep).

The experiment was concluded approximately 24 h after the long enclosures were divided (March 28). All enclosures were removed from the lake through the offshore trench. As the long enclosures were withdrawn from the

TABLE 2. Chemical characteristics of hatchery and Woods Lake water sampled during pH depressions caused by snowmelt in 1990. When mean chemical values for a location are presented, the range is given in parentheses. Brook trout fry were placed into enclosures March 17. The study was concluded on March 28.

Date/location	pH	Ca (mg/L)	Total Al (mg/L)
March 16			
Hatchery	7.05	9.96	0.09
0.9 m	5.58	—	—
2.5 m	6.59	—	—
March 18			
0.9 m	4.91 (4.73, 5.03)	2.27 (1.73, 2.81)	1.02 (0.88, 1.16)
2.5 m	6.29 (6.24, 6.34)	6.76 (6.51, 7.01)	0.48 (0.44, 0.52)
March 23			
0.9 m	5.11 (4.81, 5.29)	2.01 (1.97, 2.05)	1.38 (1.16, 1.60)
1.0 m	4.80	1.88	1.01
1.5 m	6.06	4.71	0.86
2.0 m	5.75	4.19	0.89
2.5 m	6.26 (6.25, 6.27)	6.60 (6.20, 6.99)	0.46 (0.44, 0.49)
March 28			
0.7 m	4.89	2.52	1.26
1.1 m	5.88	4.33	0.49
1.3 m	6.17	5.14	0.52
1.4 m	6.19	5.48	0.43
2.3 m	6.33	6.04	0.49

lake, each section was cut open consecutively and the fish were removed. Live and dead fish recovered from the shallow, middle, and deep sections of the enclosures were counted and placed in separate containers. Twenty live fry of each strain were collected from short–deep enclosures and shallow sections of the long enclosures to determine dry weights and ion content. In addition, fry of each strain collected from the middle and deep sections of the long enclosures were pooled and a sample of 20 fry of each strain was collected to determine dry weights and ion content.

Water Chemistry Collection and Analysis

To determine the timing and magnitude of pH depressions, water was sampled from the study area at the time of stocking, two times during the study, and at the conclusion of the study. Water samples were collected from a point approximately 5.0 to 10 cm above the lake bottom. These samples were taken through the ice at the corners of the study area and at four sites that bisected the sampling area (see Fig. 1 for locations). The first 500 mL of water pumped through the samplers was discarded to ensure that only water from the lake bottom was sampled. pH was measured with a pH meter within 4 h of sample collection. Total Al and related Ca concentrations were determined with inductively coupled argon plasma spectroscopy (ICP) analysis.

Dissolved oxygen (DO) levels and water temperature were monitored throughout the study with an oxygen/temperature meter. Discharge of the two main lake tributaries and lake level were monitored at United States Geological Survey gaging stations.

Whole-Body Ion Analysis

To determine the effects of acidic conditions on the whole-body ion content of exposed fry, 20 fry of each strain were collected from the hatchery before stocking, from the hatchery at the end of the study, from the short–deep enclosures, from shallow sections of the long enclosures, and from deeper sections (pooled from middle and deep sections) of the long enclosures. Fish were rinsed with deionized water, placed in individual plastic sampling tubes, and immediately frozen in liquid nitrogen. Individual fish were ashed for 6 h at 450°C and weighed (dry ash weight). The ashed sample was then combined with 2.0 mL of redistilled HNO_3 and 0.25 mL of ultrapure $HClO_4$ and heated for 1 h at 100°C and further digested at 180–200°C for approximately 2 h until clear. The digested samples were then ICP processed.

Statistical Procedures

The distributions of the fry between the shallow and deep sections of each long enclosure were compared with chi-square tests. A two-way contingency test was used to detect differences in the distributions of the strains between the enclosures. Analysis of variance (ANOVA) and t-tests were used to compare dry weights of fry. Linear regression analysis was used to evaluate the relationship between dry weights of fry and Ca concentrations. Significant differences were recognized at the 0.05 level ($P \leq 0.05$). Upper and lower 95% confidence interval limits are reported in the results section as ± values.

Results

Nearshore Acidification

Snowmelt events throughout the study period created a gradient of conditions from lethal low pH and high total Al in the shallows to nonlethal water conditions of moderate pH and low total Al in the deeper areas (Table 2; Fig. 1).

The disappearance of snowpack resulted in a 40-cm decrease between March 18 and 29 in lake level and large fluctuations in discharges in a tributary on the opposite shore from the study site (Fig. 2). The pH at the shallowest water samplers (<0.75 m) ranged from 4.73 to 5.29 during the study. Total Al concentrations at the shallow stations

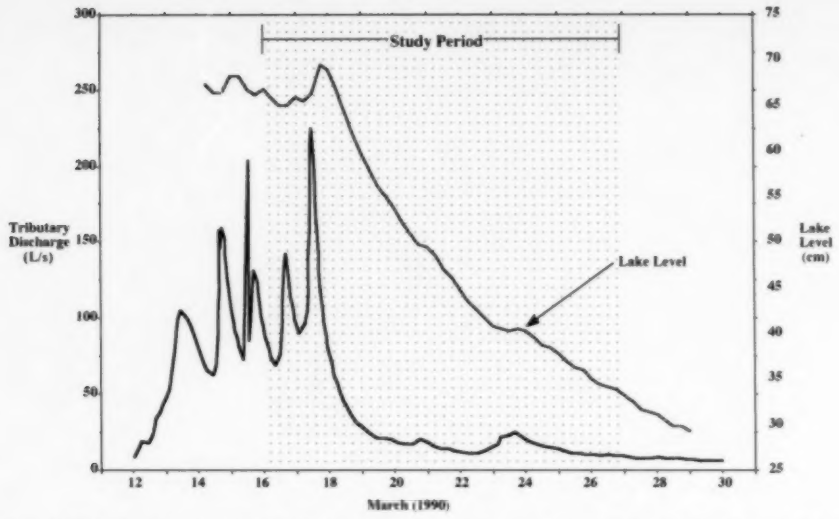

FIG. 2. Lake level and tributary discharge from one of two primary tributaries to Woods Lake measured at U.S. Geological Survey gaging stations during March 1990.

TABLE 3. Observed mean survival of Assinica (A) and Temiscamie (T) strain brook trout fry in enclosures after exposure to an episodic pH depression. Two standard errors are given in parentheses. N = the number of enclosures.

Enclosure	Strain	Day after stocking	% survival	N
Short vertical	A	6 d	0	1
	T	6 d	0	1
Short–shallow	A	12 d	0 (0)	3
	T	12 d	0 (0)	2
Short–deep	A	12 d	78 (24)	3
	T	12 d	99 (2)	2
Long	A	12 d	86 (6)	3
	T	12 d	94 (2)	2

were >1 mg/L and toxic monomeric Al species were dominant at these low pH levels (Gubala et al. 1991; see also Driscoll et al. 1980). Although total Al was relatively high at deeper stations, monomeric Al concentrations were probably <0.1 mg/L (Gubala et al. 1991). When the long enclosures were removed, the pH at their shallow end was 4.89. In the deeper, offshore region of the study area (>2.0 m), pH remained above 6.0 during the entire study period. The interface between lethal acidic and nonlethal circumneutral conditions was located probably between 1.0 and 1.5 m at the beginning of the study and between 0.7 and 1.0 m at the conclusion of the study. DO concentrations adjacent to the lake bottom remained near saturation throughout the study period (13.6–14.0 mg/L).

Survival

No fry survived in the short–shallow or short vertical enclosures in contrast with the high survival of both strains in the short–deep and long enclosures (Table 3). All fry were dead in the short vertical enclosures after 6 d of exposure. An average 78 ± 24% of the Assinica and 99 ± 2% of the Temiscamie fry were alive in the short–deep enclosures at the end of the study. Fourteen Assinica fry found dead in the sealed opening of one short–deep enclosure were presumed trapped in the enclosure opening at time of stocking. These fry were not included in the Assinica survival estimates. Survival in the long enclosures ranged from 80 to 96% for both strains.

No differences in survival between strains were observed in the long enclosures ($P > 0.2$; Table 3). An average 86 ± 6% of the Assinica fry survived compared with 94 ± 2% of the Temiscamie fry. Total recovery of fry, dead and alive, ranged from 91 to 98% of the number originally stocked in each enclosure. If fry were distributed evenly (20 fry/m) in long enclosures and the water was lethally acidic to a depth of 1 m, then the number of fry that survived in long enclosures (80–96%) was higher than expected (67–77%) in each enclosure.

Fry Distribution in Long Enclosures

Brook trout fry were not distributed evenly within long enclosures at the end of the study (Fig. 3). Significantly more live fry were found in the middle and deep sections combined (>1.1 m) than in shallow sections ($P < 0.001$). Averages of 29.8 Assinica and 27.3 Temiscamie fry/m were recovered from the combined middle and deep sections of long enclosures compared with only 4.5 Assinica and 3.1 Temiscamie fry recovered/m from shallow sections. More dead fry were recovered from shallow sections than deep sections of long enclosures ($P < 0.001$). Seventy of 76 dead Assinica (92%) and 15 of 23 (65%) dead Temiscamie fry were recovered from shallow sections of long enclosures.

More fish were recovered from middle sections than deep sections of long enclosures ($P < 0.01$; Fig. 3). Averages of 35 Assinica and 29 Temiscamie fry/m were recovered from middle sections of long enclosures, while 21 Assinica and 16 Temiscamie fry/m were recovered from deep sections. Fry distribution within each enclosure was similar among the five enclosures ($P > 0.05$).

Whole-Body Ion Content

At the end of the study, whole body Ca of fry increased,

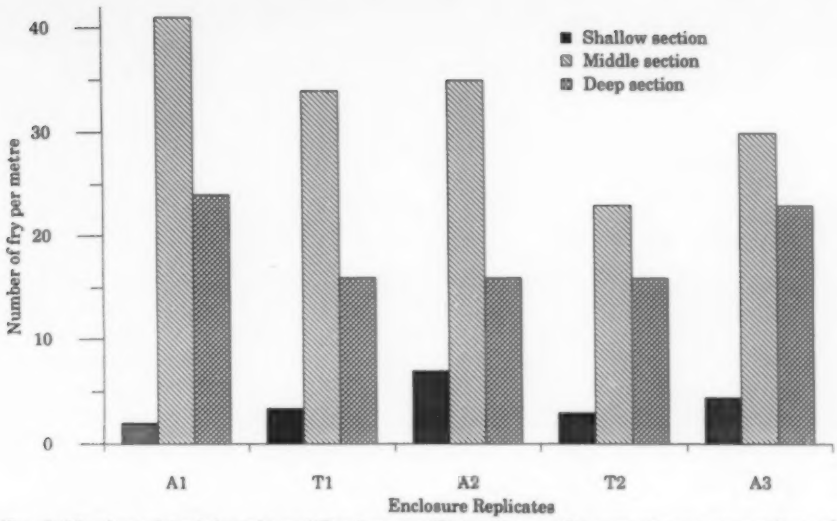

FIG. 3. Number of Assinica (A) and Temiscamie (T) brook trout fry present per metre at the end of the study in the shallow, middle, and deep sections of each long enclosure.

TABLE 4. Whole-body concentrations of Ca, Na, and K based on dry weights of Assinica (A) and Temiscamie (T) fry sampled from the hatchery and enclosures at the beginning and end of the study. Two standard errors of each estimate are given in parentheses. Percent change is expressed for each strain relative to concentrations in hatchery fish at the start of the study. $N = 20$ in all cases.

		Ca		Na		K	
Fry sample	Strain	μg/g	% change	μg/g	% change	μg/g	% change
Hatchery	A	10.92 (0.62)	—	8.27 (0.52)	—	19.80 (0.36)	—
at start	T	9.46 (0.44)	—	8.48 (0.36)	—	20.91 (0.44)	—
Hatchery	A	14.51 (1.38)	33	9.96 (0.66)	20	19.61 (0.72)	−1
at end	T	10.76 (1.02)	14	7.93 (0.48)	−7	18.81 (0.62)	−10
Short–deep	A	14.41 (0.50)	32	9.39 (0.54)	14	19.93 (0.46)	1
enclosure	T	11.82 (0.44)	25	9.10 (0.36)	7	19.57 (0.56)	−6
Long enclosure,	A	12.30 (0.32)	13	7.37 (0.44)	−11	17.71 (0.48)	−11
deeper sections	T	11.14 (0.66)	18	8.20 (0.46)	−3	18.56 (0.54)	−11
Long enclosure,	A	13.23 (0.58)	22	7.94 (0.42)	−4	18.18 (0.54)	−8
shallow section	T	10.84 (0.70)	16	8.30 (0.54)	−2	18.75 (0.38)	−10

while Na and K concentrations either increased or decreased relative to concentrations at the start (Table 4). No strain-specific changes in ion concentration occurred. Ca levels in both strains were significantly greater in fry from the hatchery and enclosures at the end of the study than in fry at the beginning of the study. Ca levels in Assinica fry from both the hatchery and short–deep enclosures at the end of the study were similar and significantly greater than in fry collected from long enclosures. In Temiscamie fry, Ca levels were similar among all fry sampled at the end of the study. Increases in Ca ranged from 13% in Assinica fry from long enclosures to 33% in hatchery Assinica fry at the end of the study. No association between Ca levels and dry weights of fry was identified through regression analysis ($P > 0.05$).

Whole-body Na levels in fry both increased and decreased relative to the concentration at the start of the study (Table 4). Na levels in Assinica fry increased the most in samples from the hatchery at the end of the study (+20%) and from the short–deep enclosures (+14%; Table 4). Na levels decreased slightly in fry sampled from shallow sections of deep enclosures (−2 to −4%). Na levels were lowest in Assinica fry sampled from the deep sections of long enclosures and significantly lower than in those sampled from the hatchery at the start (11% decrease). Temiscamie fry from short–deep enclosures had significantly higher Na levels than those sampled at the beginning of the study (+7%).

K levels in Assinica and Temiscamie fry declined 8–11% in long enclosures and changed little in short–deep enclosures when compared with those in fry at the beginning (Table 4). Assinica fry from the short–deep enclosures exhibited significantly greater K levels than fry from long enclosures, and levels were similar to those in hatchery fry at the start and end of the study period. In Temiscamie fry, K levels were similar among all fry sampled at the end of the study and were significantly lower than in hatchery fry sampled at the start (−6 to −10%).

Discussion

Brook trout fry survived in Woods Lake during episodic pH depressions when given the opportunity to move from shallow acidified waters to deeper circumneutral waters (Table 3). The uneven distributions of fry between shallow

and deep sections of long enclosures and the higher survival than expected if the fry had been evenly distributed suggested that most fry remained out of acidified nearshore waters. In laboratory investigations, brook trout alevins and juveniles avoided acidified waters, especially when test waters had high Al concentrations (Gunn and Noakes 1986; Pedder and Maly 1986). A similar avoidance response to low pH and high Al has been shown in the laboratory for lake trout (*Salvelinus namaycush*) alevins (Gunn et al. 1987). No field investigations previously have focused on the response of brook trout fry to acidified waters.

The fry may have been influenced by factors other than acidic conditions. Gradients along the length of long enclosures did occur for water temperature, light intensity, and CO_2. Water temperature ranged from 0 to 4°C and exhibited a 2°C gradient, with the shallow ends of long enclosures colder than the deep ends. The potential effect of the 2°C temperature gradient on fry movement in this investigation could not be evaluated because preferences of brook trout fry over this temperature range are unknown. Light intensity presumably was greatest at the shallow and least at the deep end of the long enclosure. Brook trout alevins typically shift from photonegative to photopositive behavior as they get older (Carey and Noakes 1981; Godin 1982; Nunan and Noakes 1985). Thus, brook trout fry in enclosures should have been attracted to the shallower depths under the ice. This interpretation corresponds with our experience electrofishing in circumneutral lakes which indicates that fry typically remain in shallow waters (0.5 m) into late spring (early June). However, low densities of fry were recovered from the shallowest section of the long enclosures (Fig. 3). High P_{CO_2} has elicited avoidance responses in Arctic char (*Salvelinus alpinus*) (Jones et al. 1985). However, P_{CO_2} in Woods Lake generally increased with depth (Gubala and Driscoll 1991) which likely would have favored a shallow distribution of fry, opposite to that observed. Although some of these variables may have contributed to fry distribution in our long enclosures, the most reasonable interpretation was that low pH and high Al concentrations were the variables that triggered an avoidance response and caused fry to seek deeper waters and to survive.

The analysis of the ion content of fry provided evidence that fry in the long enclosures did not suffer any long-term sublethal effects from their limited exposure to the acidic conditions (Table 4). Whole-body ions are expected to decline after sublethal exposure to acidic conditions (Wood et al. 1990). In our investigation, Ca concentrations actually increased among fry in the enclosures at the end of the study over fry from the hatchery at the start (Table 4). Na and K levels decreased by a maximum of only 11% (Assinica fry, long enclosures) when compared with hatchery fry at the start. Interpretation of the ion content data was difficult because of the unknown influence of original rearing conditions, the short exposure time to acidified water, a possible lack of food in the enclosures, changes in weight, and changes in body composition due to development. The small declines in Na and K suggest that fry in the long enclosures may have been negatively affected by acidic conditions; however, the increased Ca levels in the fry and the moderate water Ca levels (see Wood et al. 1990) indicate that permanent injury was unlikely.

No consistent differences between the Assinica and Temiscamie strains were observed in survival, distribution, or ion composition. A genetic component to acid tolerance has been reported in brook and brown trout (*Salmo trutta*) strains (Gjedrem 1976; Robinson et al. 1976; Swarts et al. 1978). However, in a study that compared a hybrid strain (Temiscamie × domestic) against Washington strain brook trout, no substantial differences in survival, weight, and gill histology were reported between 1-yr-old brook trout exposed for 28 d to 14 combinations of pH, Al, and Ca (Ingersoll et al. 1990a). Additional investigations are needed to quantify whether a differential physiological tolerance or avoidance behavior exists between these and other strains to acidic conditions. Differences in behavior among strains in response to acidic conditions could be as important to survival as physiological tolerance. For example, a strain physiologically tolerant to low pH could exhibit a weaker avoidance response to acidic conditions in the wild than a strain less physiologically tolerant. In this case, the physiologically less tolerant strain may more rapidly sense and avoid acidic conditions than a more tolerant strain and thus avoid lethal exposure.

This investigation and others help explain why lake trout populations characteristically suffer recruitment failures earlier than do brook trout in lakes susceptible to episodic acidification. Brook trout typically spawn earlier and take less time to hatch than lake trout (Power 1980). Thus, under natural conditions, brook trout occur as free-swimming fry when lake trout are still relatively immobile alevins. In addition, lake trout alevins have been reported to prefer to remain near substrate rather than exhibit an avoidance response to acidic conditions (Gunn et al. 1987). Thus, the temporal relationship between the life stage present and acidified snowmelt combined with different avoidance responses of the species favors survival of brook trout over lake trout in waters that experience episodic acidification. Comparative bioassays with brook trout and lake trout fry in acidic water also have indicated significantly lower mortality rates for brook trout (Grande et al. 1978).

In summary, brook trout fry of two strains were observed to survive in nearshore areas of a lake during episodic pH depressions when they had access to deeper circumneutral waters. The probable mechanism that caused the survival of these fry was an avoidance response to acidic conditions and subsequent movement to deeper waters. Mortality of fry contained in the short–deep and long enclosures was probably higher than would have occurred in fish afforded full freedom of movement; thus, the estimates of survival were likely conservative and lower than in reality. However, the enclosures used in the study provided a practical means to evaluate the behavior of fish exposed to a natural episodic pH depression. Additional investigations with this type of enclosure could be conducted with other life stages of brook trout, other species, and different strains to help determine behavior of fish exposed to natural acidic conditions. Field studies of longer duration are needed to better evaluate and understand the effects of pH depressions on growth, fish behavior, and ion regulation.

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Why Are the PCB Concentrations of Salmonine Individuals from the Same Lake So Highly Variable?[1]

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Madenjian, C.P., S.R. Carpenter, and P.S. Rand. 1994. Why are the PCB concentrations of salmonine individuals from the same lake so highly variable? Can. J. Fish. Aquat. Sci. 51: 800–807.

An individual-based model (IBM) was applied to the Lake Michigan rainbow trout (*Oncorhynchus mykiss*) population, with the objectives of explaining the observed variation in growth and in polychlorinated biphenyl (PCB) concentration within the population. When variation in prey PCB concentration was incorporated into the model, variability in PCB concentration among individual rainbow trout was fully explained by the IBM. Although number of spawnings and number of years spent in a stream prior to first entering the lake were factors in determining growth, these life history characteristics appeared to have only a minor impact on PCB accumulation rate in rainbow trout. The IBM application to the rainbow trout population was compared with an application to the Lake Michigan lake trout (*Salvelinus namaycush*) population. Modeling results indicated that the lower observed PCB concentrations in rainbow trout compared with lake trout were chiefly due to greater longevity in lake trout. The IBM simulations identified gross growth efficiency, assimilation efficiency of PCBs from food, and diet as other important sources of variability in salmonine PCB concentrations.

Un modèle fondé sur des cas individuels (« individual-based model » : IBM) a été appliqué à la population de truites arc-en-ciel (*Oncorhynchus mykiss*) du lac Michigan; l'objectif était d'expliquer la variation observée du taux de croissance en fonction de la concentration en biphényles polychlorés (BPC) au sein de la population. Lorsque la variation de la concentration en BPC dans les tissus des proies est incorporée dans le modèle, on observe que la variabilité de la concentration en BPC chez des truites arc-en-ciel prises individuellement, est entièrement expliquée par l'IBM. Même si le nombre de pontes et le nombre d'années passées dans un cours d'eau avant la première présence dans le lac constituent des facteurs qui interviennent dans la croissance, il demeure que ces caractéristiques du cycle biologique paraissent n'avoir qu'un effet mineur sur le taux d'accumulation des BPC dans les tissus de la truite arc-en-ciel. L'application de l'IBM à la population de truites arc-en-ciel a été comparée à son application à la population de touladis (*Salvelinus namaycush*) du lac Michigan. Les résultats de la modélisation montrent que la concentration inférieure en BPC qui est observée dans les tissus de la truite arc-en-ciel, à comparer à celle du touladi, est essentiellement attribuable à la plus grande longévité de ce dernier. Les simulations par IBM ont montré que l'efficacité brute de la croissance, que l'efficacité de l'assimilation des BPC à partir des aliments ainsi que le régime alimentaire constituent d'autres sources importantes de la variabilité de la concentration en BPC chez ces espèces.

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Polychlorinated biphenyls (PCBs) are a focus of societal concern, management activity, and research in the Great Lakes and other ecosystems (National Research Council 1986; Evans 1988; Levin 1989). Accumulation of PCBs by Great Lakes salmonines may pose a health threat to people consuming these fish (Cordle et al. 1982; Fein et al. 1984) and may impede successful natural reproduction by certain salmonine species (Mac 1988). Because of biomagnification through the food chain, salmonines tend to exhibit relatively high PCB levels (Weininger 1978). Consequently, the Michigan Department of Natural Resources (1991) and Wisconsin Department of Natural Resources (Masnado 1987) have issued consumption advisories for Lake Michigan salmonines.

Considerable variation in PCB concentrations within and among Lake Michigan salmonine populations has been documented by Masnado (1987) during a 1985 survey. Lake trout (*Salvelinus namaycush*) showed the highest PCB levels (average PCB concentration of 4.1 mg/kg) of all the salmonines occurring in the lake whereas rainbow trout (*Oncorhynchus mykiss*) exhibited the lowest PCB levels (average PCB concentration of 1.3 mg/kg). A modeling comparison of PCB accumulation in lake trout with that in

[1]Contribution 843 of the National Biological Survey, Great Lakes Center, 1451 Green Road, Ann Arbor, Mich.

rainbow trout would provide insight on mechanisms underlying the interspecific and intraspecific variation in PCB concentrations of salmonines.

Within a particular salmonine population in Lake Michigan, sources of variation that contribute to variability in PCB concentrations include (1) differences in diet within a cohort (fish of the same age) due to fish size (i.e., larger predators consume larger prey fish), (2) differences in age, and (3) differences in dietary PCB from spatial, behavioral, and physiological differences among individuals of the population. Madenjian et al. (1993) used an individual-based model (IBM) to describe the variability in PCB levels within the Lake Michigan lake trout population. The individual-based modeling approach was particularly well suited to address the first source of variation mentioned above, and the IBM could easily be adapted to address the other two sources of variation as well. The third source of variation was critical in explaining the variability in lake trout PCB levels (Madenjian et al. 1993).

Another potential source of PCB variability, which is particularly applicable to rainbow trout, is from variability in life history characteristics. Seelbach (1993) observed significant differences in sizes of Lake Michigan rainbow trout attributable to differences in number of spawnings and to number of years spent in the stream (or hatchery) before first entering the lake. Perhaps the differences in these life history characteristics were major contributors to the variability in the rainbow trout PCB concentrations.

Our objectives for this study were to (1) develop an IBM for PCB accumulation within the rainbow trout population in Lake Michigan, (2) using the IBM, investigate how PCB accumulation rate varies across the rainbow trout life history forms, (3) compare the results of the IBM application to rainbow trout with those for lake trout from the study by Madenjian et al. (1993), and (4) use the modeling results to identify the important factors leading to the differences in PCB concentrations between the two salmonine species.

Model

We developed an IBM for rainbow trout growth and accumulation of PCBs that was similar to the IBM developed by Madenjian et al. (1993) for lake trout (Fig. 1). The bioenergetics module was based on the Rand et al. (1993) model, which was specifically developed for Lake Michigan rainbow trout.

We applied the same PCB accumulation submodel that Madenjian et al. (1993) used in their lake trout IBM to the rainbow trout IBM, with only two differences. Madenjian et al. (1993) used a PCB assimilation efficiency from food of 0.8 for lake trout. Similarly, Jensen et al. (1982) and Thomann and Connolly (1984) used a value of 0.8, and Borgmann and Whittle (1992) used a value of 0.75, for modeling lake trout PCB accumulation. However, because PCB accumulation in rainbow trout has not been previously modeled, we adjusted the efficiency of PCB assimilation from food in order to match the observed PCB concentrations in rainbow trout. The PCB excretion term of the submodel depends, in part, on the average lipid concentration, ρ, of the predator. ρ was set equal to 0.155 in the lake trout IBM (Madenjian et al. 1993) whereas ρ was assigned the value of 0.085 in the rainbow trout IBM, based on data from studies by Niimi (1979) and Niimi and Cho (1981).

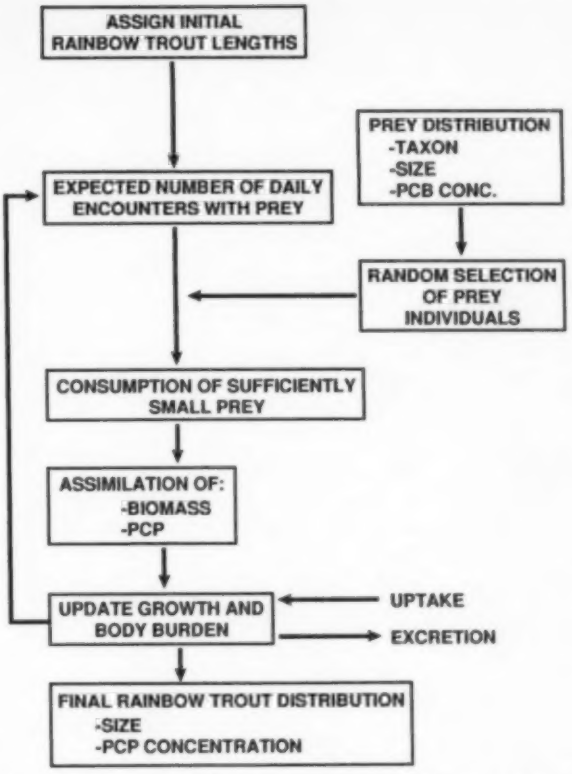

FIG. 1. Diagram of IBM for accumulation of PCBs by rainbow trout.

On the average, rainbow trout lose 2.7% of their PCB body burden during spawning (Niimi 1983). In our simulations, rainbow trout spawned on April 30; each spawning fish lost 10% of its weight (Rand et al. 1993) and 2.7% of its PCB body burden on that day.

The predator–prey encounter model was similar to the one used in the lake trout IBM (see Madenjian et al. 1993 for details). See Appendix Table A1 for an example of the encounter rates used in one of the rainbow trout IBM simulations.

Lake Michigan Data

Seelbach (1993) concluded that adult size of rainbow trout in Lake Michigan was best characterized as a function of not only age, but also the number of years spent in the lake and the number of times the fish has spawned. Most naturally spawned rainbow trout spend 2 yr in a stream before entering the lake (Seelbach 1993). Some naturally spawned rainbow trout spend only 1 yr in a stream before entering the lake, and almost all hatchery fish are stocked as yearlings (Seelbach and Whelan 1988). Rand et al. (1993), in an energetics study of the rainbow trout population of Lake Michigan, divided the population into groups called life history forms. Each life history form was designated by a series of three digits. The first digit represented the age of the fish when it entered Lake Michigan, the second number was the number of birthdays the trout had spent in Lake Michigan, and the third digit was equal to the num-

ber of times the fish had spawned. The birthday for each fish was arbitrarily selected as January 1 for purposes of the nomenclature system. Thus, a 1.2.0 rainbow trout had entered the lake at age 1, had spent two birthdays in the lake, but had not spawned yet. Seelbach (1993) identified over 40 life history forms in the Lake Michigan rainbow trout population. The Michigan Department of Natural Resources has conducted creel surveys in the state's Lake Michigan waters (Rakoczy 1992). Rainbow trout were collected by anglers from February through November; length, weight, and life history form were determined for each rainbow trout. According to the survey, about 85% of the individuals in the population can be classified into one of nine life history form categories, and nearly 70% of the lake's rainbow trout had spent less than three birthdays in the lake.

The Wisconsin Department of Natural Resources determined PCB concentration in 25 rainbow trout from March through October of 1984 and 1985 (Masnado 1987). Most of the fish were captured by Lake Michigan anglers, although a few smaller fish were caught with electrofishing gear employed by staff of the Wisconsin Department of Natural Resources. Lengths and weights were recorded, and lipid and PCB concentrations were determined from skin-on fillets from each of the 25 trout (see Masnado 1987 for details on laboratory analyses). Individual PCB congener concentrations were not measured; concentrations for total PCBs were reported. PCB concentrations from skin-on fillets were lipid-corrected in order to more accurately portray the whole-body concentration (see Madenjian et al. 1993). The expected lipid proportion of whole-body weight was taken from Niimi (1979) and Niimi and Cho (1981).

PCB concentrations in Lake Michigan prey fish and in Lake Michigan water were taken from Madenjian et al. (1993). Terrestrial insects comprise a substantial portion (approximately 67%) of the macroinvertebrate portion of the rainbow trout diet (M. Miller, Wisconsin Department of Natural Resources, Madison, Wis, unpublished data). To the best of our knowledge, PCB concentrations in terrestrial insects have not been measured. We expect that PCB concentrations in terrestrial insects are minimal, so we assigned a value of 0 mg/kg to that diet component. Hence, the macroinvertebrate PCB concentrations for the rainbow trout diet were estimated by dividing the macroinvertebrate PCB concentrations in the lake trout diet by 3. Zooplankton PCB concentrations were taken from Evans et al. (1991). We assumed that PCB concentrations in zooplankton showed a slight decline from 1980 through 1985, as did aquatic macroinvertebrate concentrations (Madenjian et al. 1993). PCB concentration for Lake Michigan zooplankton was assigned the value of 0.04 mg/kg for 1980 and 1981 and was 0.03 mg/kg for 1982–85.

IBM Application

First, the rainbow trout IBM was calibrated with growth data by matching observed with predicted length–frequency distributions for eight of the nine most common life history forms. The fish from the ninth life history form, 1.0.0, were not fully recruited to the fishery and therefore their growth could not be directly calibrated. We calibrated the growth of simulated rainbow trout with sizes of rainbow trout observed during the fall. The "average" day of autumn at which rainbow trout length–frequencies were observed was October 10; thus, simulations were completed on that day of the appropriate simulation year. The IBM was fitted to the observed length–frequency distributions by adjusting the encounter rates for each of the prey fish categories. Diet survey data provided rough estimates of the encounter rates, but the rates were further adjusted by trial and error. Accuracy of the predicted length–frequency distribution was based on the difference between observed and predicted mean lengths of the cohort and on the sum of the squares of the differences between observed and predicted numbers of rainbow trout in each of the length intervals. Initial lengths of the 500 simulated rainbow trout individuals depended on life history form; those fish entering the lake as 2-yr-olds were larger than fish entering the lake as yearlings. Initial lengths were assigned based on measurements of age-1 and age-2 rainbow trout immediately prior to entering the lake (P. Seelbach, Michigan Department of Natural Resources, Ann Arbor, Mich., unpublished data). Simulations began on May 1, which is a typical date of lake entry by rainbow trout in Lake Michigan (Rand et al. 1993).

TABLE 1. Life history forms of simulated rainbow trout individuals used to calibrate the IBM with observed PCB levels from 25 rainbow trout collected from Lake Michigan during 1984 and 1985 (see text for details on IBM).

Last year of simulation	Life history forms of simulated rainbow trout individuals
1984	1.0.0, 1.1.0, 1.1.0, 1.1.0, 1.2.0, 1.2.0, 1.2.0
1985	1.0.0, 1.0.0, 1.1.0, 1.1.0, 1.1.0, 1.1.0, 1.2.0, 1.2.0, 1.2.0, 1.2.0, 1.2.0, 1.3.1, 1.3.1, 1.3.0, 1.2.1, 2.1.0, 2.2.0, 1.4.2

Next, the contaminant accumulation submodel of the rainbow trout IBM was calibrated with the 1984 and 1985 Wisconsin Department of Natural Resources data set for PCB determinations (lipid-corrected values) in 25 individual rainbow trout. PCB calibration required a series of steps. No age data were available for individuals measured for PCB concentrations. Judging from the lengths, one fish in 1984 and two fish in 1985 were age 1. The remaining 6 and 16 rainbow trout from 1984 and 1985, respectively, were assumed to show a similar distribution with respect to age as shown by the rainbow trout surveyed by the Michigan Department of Natural Resources from 1983 through 1990 (R. Hay, unpublished data). This assumption appeared reasonable because all of the fish surveyed by the Michigan Department of Natural Resources and almost all of the rainbow trout collected by the Wisconsin Department of Natural Resources for contaminant analyses were captured by anglers. We assumed that the PCB body burden of rainbow trout at the time of first entry into Lake Michigan was zero. This assumption seemed justified because measurements on both hatchery and wild fish just prior to initial entry into Lake Michigan showed that PCB levels in these fish were below the detection limit of 0.20 mg/kg (J. Amrhein, Wisconsin Department of Natural Resources, Madison, Wis., personal communication).

To match the 25 observations (during 1984 and 1985) of PCB concentration in Lake Michigan rainbow trout, we sam-

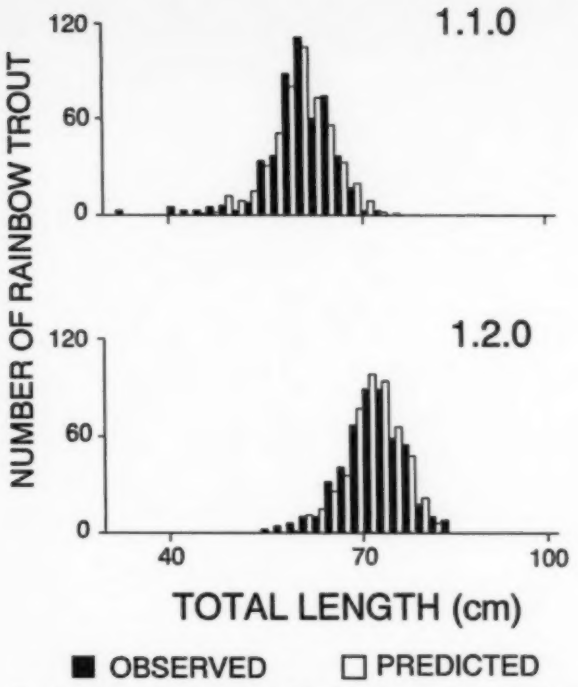

FIG. 2. Observed and predicted length–frequency distributions for life history forms 1.1.0 and 1.2.0 of rainbow trout from Lake Michigan. Observed (black bars) distributions were based on rainbow trout caught in Lake Michigan from 1983 through 1990 (R. Hay, unpublished data). Predictions (white bars) were based on an IBM for rainbow trout growth. For each age group, a cohort of 500 observed fish was compared with a cohort of 500 simulated fish.

pled 25 individuals from a simulated Lake Michigan rainbow trout population in the following manner. For 1985, we simulated nine different cohorts corresponding to the nine most common life history forms for 1985. For 1984, the two most common life history forms plus the 1.0.0 form were simulated. Each cohort, except for the 1.0.0 cohorts, was simulated until July 15, 1985, which was the "average" sampling day for the Masnado (1987) salmonid PCB sampling study. The 1.0.0 cohorts were grown until October 10 because October was the time of capture for the fish believed to be 1.0.0 rainbow trout. Rainbow trout individuals were randomly selected from the simulated cohorts, each of which comprised 500 individuals, using the schedule presented in Table 1.

As in the lake trout IBM, the prey PCB variation factor for the rainbow trout IBM was adjusted to explain the observed variation in rainbow trout PCB concentrations. The degree of variability in PCB concentrations of the prey was controlled by adjusting the prey PCB variation factor (see Madenjian et al. 1993 for more details on the prey PCB variation factor).

Results

Observed and predicted length–frequency distributions were in reasonably good agreement for the two most common life history forms of rainbow trout caught by Lake Michigan anglers: life history forms 1.1.0 and 1.2.0 (Fig. 2).

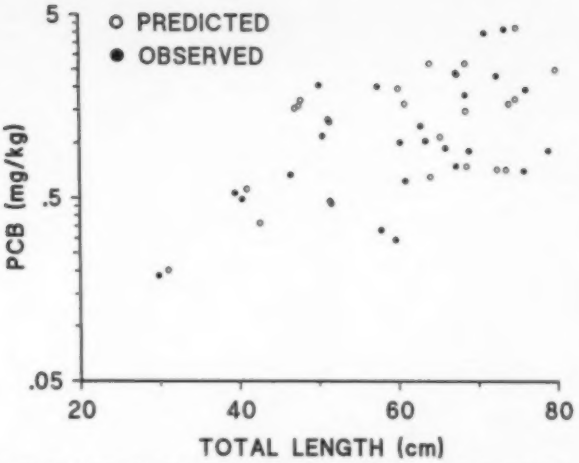

FIG. 3. Observed and predicted whole-fish PCB concentrations versus total length of rainbow trout from Lake Michigan in 1984 and 1985. Observations were from rainbow trout sampled by the Wisconsin Department of Natural Resources (M. Miller, unpublished data). Predictions were based on an IBM for accumulation of PCBs by rainbow trout, using a prey PCB variation factor equal to 60 (see IBM Application section).

Furthermore, predicted and observed distributions were well matched for the next six most common life history forms.

Good agreement between observed and predicted PCB concentrations was obtained when the prey PCB variation factor was equal to 60 (Fig. 3). In other words, when the extreme groups in a particular rainbow trout age cohort were subjected to a 60% deviation from the scheduled prey PCB concentrations (the scheduled prey PCB concentrations are presented in appendix 2 of Madenjian et al. 1993), predicted and observed variances about the respective regression lines were similar. The observed variance about the regression line was 0.417 whereas the predicted variance was 0.374; these variances were not significantly different from one another. Furthermore, the assimilation efficiency of PCBs from food was set equal to 0.5 for rainbow trout feeding to achieve this fit between observed and predicted PCB levels.

The IBM predicted that an extra year in the stream before first entering the lake led to a slight increase in PCB concentration after 1 or more yr of residence in the lake. Average PCB concentration for the 2.1.0 form was slightly higher than that for the 1.1.0 form (Table 2). Similarly, mean PCB concentration for the 2.2.0 form was slightly higher than the mean concentration for the 1.2.0 form (Table 2). The model also indicated that spawning led to a slight increase in PCB concentration (Table 2). Overall, number of years in the stream (or hatchery) before first entry into the lake and number of spawnings had relatively minor effects on PCB accumulation rate.

Rainbow trout grow appreciably faster than lake trout (Fig. 4). For the following comparisons, time in streams for spawning by rainbow trout was considered time in the lake. Furthermore, means and standard deviations for the rainbow trout population were calculated over the first 4 yr in the lake by weighting the eight most common life history forms (other than 1.0.0 fish) in proportion to their occurrence in the population. According to the IBMs, on the average, lake

TABLE 2. Predicted PCB concentrations in various life history forms of rainbow trout from Lake Michigan, mid-July 1985. Each mean PCB concentration is based on an IBM-simulated cohort of 500 individuals (see text for details on IBM). Standard deviation is enclosed in parentheses.

Life history form	Mean PCB concentration (mg/kg)
1.1.0	1.03 (0.45)
2.1.0	1.13 (0.49)
1.2.0	1.58 (0.66)
2.2.0	1.65 (0.68)
1.2.1	1.71 (0.71)
1.3.0	2.25 (0.92)
1.3.1	2.32 (0.95)

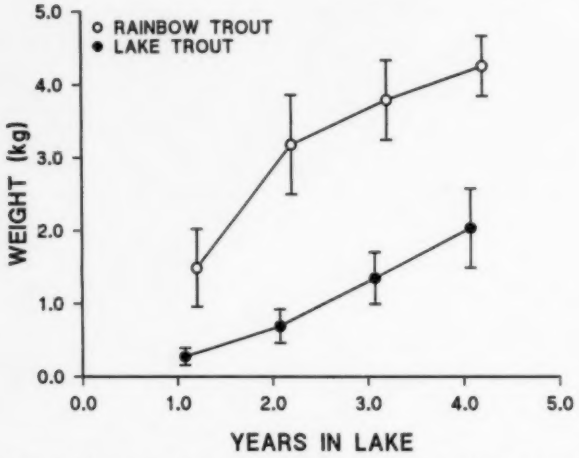

FIG. 4. Mean individual weight for lake trout and rainbow trout populations in Lake Michigan as a function of years in the lake, as predicted by IBMs. Bars represent 1 standard deviation from the mean.

trout and rainbow trout accumulated PCBs at similar rates during their first 2 yr in the lake (Fig. 5). During the next 2 yr, rainbow trout accumulated PCBs at a slightly higher rate. Average PCB concentration was somewhat higher for rainbow trout prey than for lake trout prey during the first 2 yr in the lake (Fig. 6). This difference could be indirectly attributed to the rainbow trout's more rapid growth. Of all the prey items available to Lake Michigan salmonines, adult alewife (*Alosa pseudoharengus*) exhibit the highest PCB concentrations (Madenjian et al. 1993). Due to their more rapid growth, rainbow trout are able to consume larger alewife at an earlier age than lake trout (Fig. 7). However, the proportion of adult alewife in lake trout diet increases with age (Stewart and Ibarra 1991). By the fourth year in the lake, mean PCB concentrations in the prey were similar between the two salmonine species. By age 7, adult alewife represent roughly 80% of the lake trout diet (Stewart and Ibarra 1991).

Gross growth efficiency, which is the proportion of biomass consumed by a predator that is actually converted into predator biomass, also appears to be an important regulator of PCB accumulation in salmonines. The bioenergetics submodel incorporated in the IBMs allows gross growth efficiency to vary from individual to individual, depending on

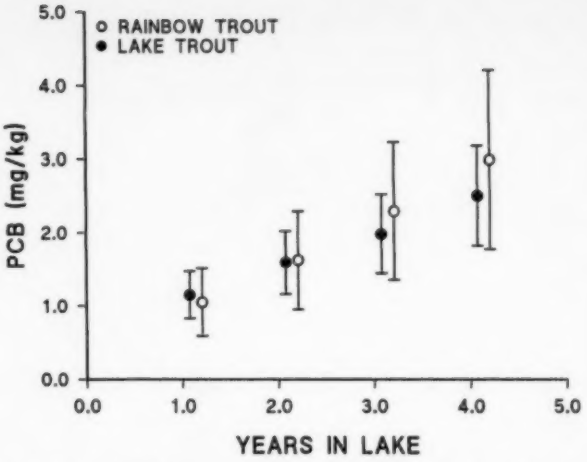

FIG. 5. Mean PCB concentrations of lake trout and rainbow trout from Lake Michigan in 1985 as a function of years in the lake, as predicted by IBMs. Bars represent 1 standard deviation.

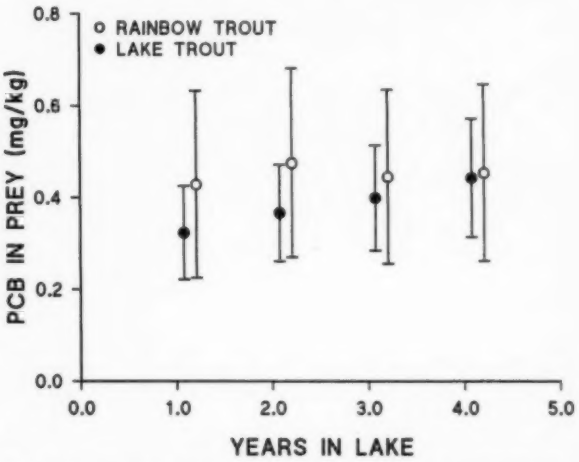

FIG. 6. Mean PCB concentrations in prey of lake trout and rainbow trout from Lake Michigan in 1985 as a function of years in the lake, as predicted by IBMs. Means were cumulative, that is, they were calculated over the entire time spent in the lake. Bars represent 1 standard deviation.

size. Gross growth efficiency represents an encapsulation or summary of the bioenergetics submodel. All other factors being equal, PCB accumulation by a predator should increase as gross growth efficiency decreases. To grow the same amount, an individual with a low gross growth efficiency must consume a greater amount of prey biomass and therefore ingest a greater amount of PCBs than an individual with a high gross growth efficiency. Initially, gross growth efficiency is similar between species (Fig. 8). However, rainbow trout gross growth efficiency decreases drastically with time.

Discussion

The rainbow trout IBM developed in this study simulated the size variability observed within the various life history

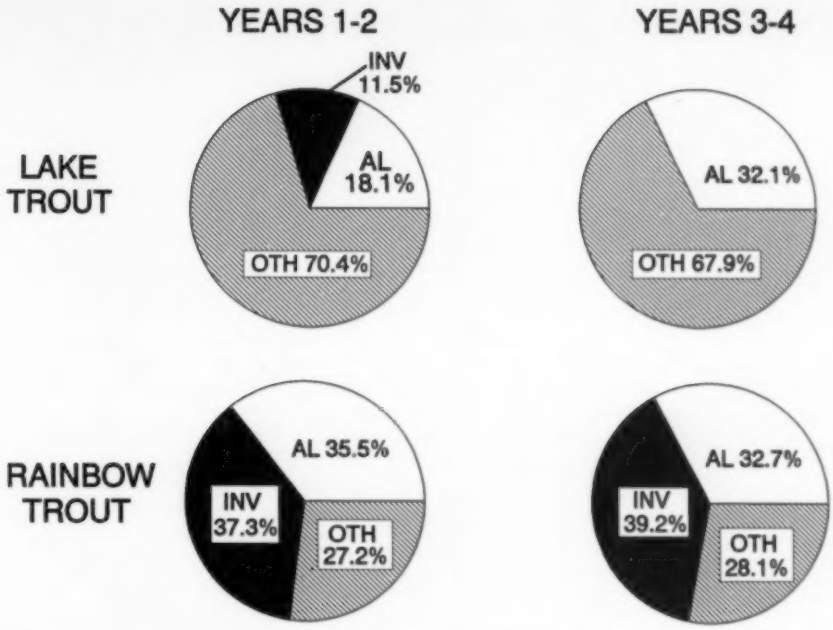

FIG. 7. General diet composition for lake trout and rainbow trout from Lake Michigan during 1980–85, as predicted by IBMs. Diet is proportioned between invertebrates (INV), yearling and older alewife (AL), and other fish (OTH).

forms of the Lake Michigan population. The IBM also simulated the variation in PCB concentrations within the Lake Michigan rainbow trout population. This variation was fully explained when a substantial amount of variability in the prey PCB concentrations was incorporated in the model. In fact, an even greater degree of variation in prey PCB concentrations was included in the rainbow trout IBM than in the IBM presented by Madenjian et al. (1993) for lake trout.

The greater variation in the prey PCB concentrations needed for the rainbow trout IBM compared with lake trout model could be attributed to the greater diversity in the rainbow trout's diet. Rainbow trout have been observed to feed on terrestrial insects floating on the water surface in the central region of the lake during July and August (R. Hay, personal communication). We would expect that terrestrial insects contain very low PCB body burdens because of their limited exposure to these chemicals. Invertebrates comprise a substantial portion of the adult rainbow trout diet whereas adult lake trout are almost exclusively piscivorous. If certain rainbow trout individuals preferred feeding on alewife while others preferred terrestrial insects, food preferences would substantially contribute toward the variation in prey PCB concentrations.

The IBM specifically takes into account differences in gross growth efficiencies between individuals of the cohort based on size differences. According to the IBM results, variation in gross growth efficiency within an age cohort is relatively small (Fig. 8); this variability could not account for all of the observed variation in rainbow trout or lake trout PCB concentrations. Consequently, variation in prey PCB concentration was imposed to calibrate the IBMs with observed PCB concentrations.

The IBM applications for lake trout and rainbow trout

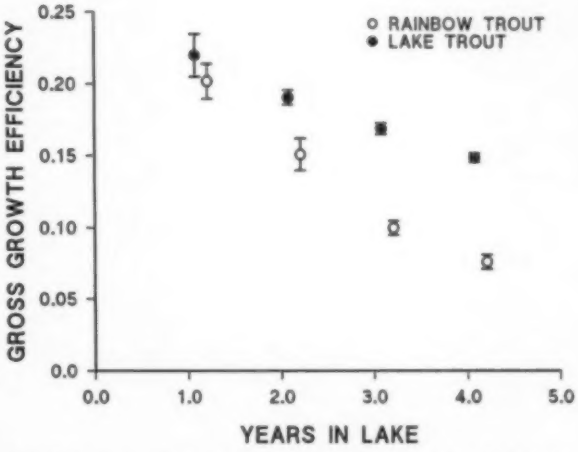

FIG. 8. Mean gross growth efficiency of lake trout and rainbow trout from Lake Michigan as a function of years in the lake, as predicted by IBMs. Means were cumulative, that is, they were calculated over the entire time spent in the lake. Bars represent 1 standard deviation.

populations suggested that rainbow trout assimilate PCBs from food with less efficiency than lake trout. For lake trout, we used an assimilation efficiency of 0.8; this same value has been used in previous PCB accumulation models for lake trout. The rainbow trout IBM predictions were in good agreement with the observed PCB concentrations when the assimilation efficiency was set equal to 0.5. This value is within the range of those determined in laboratory studies for the *Oncorhynchus* genus. Lieb et al. (1974) estimated that

juvenile rainbow trout, fed a synthetic diet which was spiked with PCBs, assimilated 50% of the ingested quantity of PCBs during the first 16 wk of the experiment and then assimilated 68% of the ingested PCBs during the next 16 wk. Adult rainbow trout, in which gelatin capsules spiked with PCBs were inserted into their stomachs, assimilated 75% of the PCBs (Niimi and Oliver 1983). Gruger et al. (1975) estimated that coho salmon (*Oncorhynchus kisutch*) juveniles retained only 30–60% of the ingested quantity of PCBs.

Assimilation efficiency of PCBs from food is poorly understood, yet modeling efforts from this study, as well as from lake trout studies (Weininger 1978; Madenjian et al. 1993), have identified this process as an important component of PCB accumulation in salmonines. The considerable variation in assimilation efficiency from the above-mentioned laboratory studies may be due to a suite of factors, including the type of food, amount of food, and frequency of feeding. The fish food pellets offered in the Gruger et al. (1975) experiment were more similar to a natural salmonine diet than the synthetic diets used in the other two studies. Clearly, more laboratory studies are needed to better understand assimilation efficiency of PCBs from food. Variation in dietary PCBs may be due, in part, to variability in assimilation efficiencies among individual fish of a cohort (see Madenjian et al. 1993). Again, very little is known about variability in assimilation efficiencies among individual fish of the same population.

The most critical life history characteristic, with regard to PCB contamination, of the three characteristics used in the nomenclature system for rainbow trout is number of birthdays in the lake. Number of years in the stream (or hatchery) before first entering the lake or number of spawnings had little impact on PCB accumulation rate, according to the IBM. The slightly higher PCB concentration in rainbow trout that had spent 2 yr in a stream before first entering the lake, as opposed to those only spending 1 yr in the stream, may be attributed to decline in gross growth efficiency with size. Rainbow trout that have spent 2 yr in a stream before entering the lake are slightly larger than those individuals residing only 1 yr in a stream before first entering the lake. Thus, those fish having spent 2 yr in a stream have a slightly lower gross growth efficiency during their duration in the lake than rainbow trout having spent only 1 yr in a stream (or hatchery). Similarly, rainbow trout that spawn during the year exhibit marginally lower gross growth efficiency, and consequently slightly higher PCB concentration, than those rainbow trout that do not spawn.

The modeling results indicated that the relatively low PCB levels observed in the Lake Michigan rainbow trout population are chiefly due to the rainbow trout's relatively early recruitment to the sport fishery. Most of the rainbow trout fished from the lake are either age 2 or age 3. Rainbow trout appear to accumulate PCBs at a slightly higher rate, on the average, than lake trout during the first 4 yr in the lake. Lake trout are first fully recruited to the sport fishery at age 5, and fish of age 7 are commonly captured by anglers. Thus, for Lake Michigan salmonines, much of the variation in PCB concentrations can be explained by fish age. Diet, efficiency of PCB assimilation from food, and gross growth efficiency may also contribute to differences in PCB accumulation between species.

The rainbow trout IBM was developed using the available age–length, bioenergetics, and contaminant data. We recommend that the model be updated as more data become available, particularly with regard to diet data and PCB observations. An intensive sampling of PCB concentrations in rainbow trout, such as the 1985 sampling for lake trout performed by the Wisconsin Department of Natural Resources, would be helpful. Additionally, PCB concentrations in skin-on fillets should be compared with those for whole fish to ensure that the lipid-correction procedure is accurate for rainbow trout.

From the perspective of reducing PCB concentration in salmonines harvested by Lake Michigan anglers, it would be advantageous to stock rainbow trout rather than lake trout because the rainbow trout's growth is rapid and harvest occurs relatively quickly (most fish are caught from 1 to 2.5 yr from time of stocking). This advantage should be weighed against the exotic species status for rainbow trout whereas the lake trout is the only salmonine native to Lake Michigan. Fishery managers may want to include other factors, such as angler preferences, in deciding upon salmonine stocking rates for Lake Michigan.

In summary, the modeling results indicated that variation in prey PCB concentrations was an important contributor to variation in PCB concentrations of both lake trout and rainbow trout in Lake Michigan. Apparently, the rainbow trout population is exposed to even greater variability in prey PCB concentrations than the lake trout population. Number of spawnings and number of years spent in the stream (or hatchery) before first entry into the lake had little effect on PCB accumulation rate in rainbow trout. The low PCB concentrations exhibited by rainbow trout, in comparison with lake trout, can be explained by longevity. Most rainbow trout caught by Lake Michigan anglers are ages 2 and 3 whereas lake trout are not fully recruited to their sport fishery until age 5. Variation between the two species was also attributable to diet, assimilation efficiency of PCBs from food, and gross growth efficiency.

Acknowledgments

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Appendix

TABLE A1. Average prey encounter rates (number of encounters per day) with prey fish for an individual rainbow trout of life history form 1.2.0 used in IBM simulations of PCB accumulation by rainbow trout in Lake Michigan. Rough estimates of the prey encounter rates were based on diet surveys by D. Jude (unpublished data) and M. Miller (unpublished data). Prey encounter rates were then adjusted so that predicted length–frequency distribution matched the observed length–frequency distribution. YOY = young-of-the-year, YRL = yearling, AD = adult. Zooplankton prop. refers to the proportion of the invertebrate diet that is composed of zooplankton.

Simulation year	Month(s)	Sculpin/ other fish	Smelt			Alewife			Zooplankton prop.
			YOY	YRL	AD	YOY	YRL	AD	
1	May–Aug.	0.3	0	0	0	0	0.5	2.0	0.10
1	Sept.–Oct.	0.2	0.2	0.3	0.3	2.0	0.1	2.0	0.15
1	Nov.–Dec.	0.3	0	0.3	0.3	0	0.1	1.0	0.15
1	Jan.–Apr.	0.9	0	0.2	0.6	0	0.3	0.5	0.15
1	May	0	0	0.2	0.2	0	0.2	3.0	0.20
2	June	0	0	0.2	0.2	0	0.2	1.0	0.80
2	July–Aug.	4.6	0	0.3	0.3	0	0.1	0.7	0.60
2	Sept.	3.2	0	0.3	0.3	0	0.2	0.7	0.60
2	Oct.	1.4	0	0.2	0.3	0	0.3	0.7	0.60
2	Nov.–Dec.	0.8	0	0.2	0.2	0	0.2	0.7	0.60
2	Jan.–Apr.	0.5	0	0.2	0.2	0	0.2	0.5	0.60
3	May	0	0	0.1	0.1	0	0.2	0.4	0
3	June	0.2	0	0.2	0.2	0	0.2	0.4	0.25
3	July–Aug.	1.2	0	0.2	0.2	0	0.2	0.4	0.60
3	Sept.	0.9	0	0.1	0.1	0	0.2	0.4	0.60
3	Oct.	0.5	0	0.1	0.1	0	0.1	0.4	0.60

Geographic Responses of Groundfish to Variation in Abundance: Methods of Detection and Their Interpretation

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Recent published studies have used data from bottom trawl surveys of groundfish populations to test whether distributional area and abundance are correlated. Two studies that used different indices to represent the distributional area of Georges Bank haddock (*Melanogrammus aeglefinus*) yielded conflicting results. To determine whether this is an example of different distributional indices measuring different things, both indices were regressed against estimates of abundance of haddock from a different but neighbouring location on the southwestern Scotian Shelf. Positive correlations were observed for immature age-classes using both indices whereas only one of the two indices resulted in positive correlations for mature age-classes. The following factors contributed to the lack of agreement among distributional indices: (1) age-aggregated indices potentially obscure correlations between distributional area and abundance for individual age-classes; (2) distributional indices that depend on the magnitude of catch rates confound variation in the large-scale horizontal distribution of stocks with diurnal variation in the three-dimensional distribution of schools; (3) distributional indices that scale positively with abundance generate spurious correlations. The results suggest that the outcome of any test of whether distributional area and abundance are correlated depends on the index chosen to represent distributional area.

Des travaux récemment parus ont fait appel à des données obtenues par chalutage de fond sur des populations de poissons de fond afin qu'on vérifie s'il existe un corrélation entre l'abondance et la surface occupée. Deux études qui ont fait appel à des indices différents afin de représenter la surface occupée par l'aiglefin (*Melanogrammus aeglefinus*) du banc Georges, ont conduit à des résultats opposés. Pour déterminer s'il s'agit d'un cas où des indices différents mesurent des phénomènes différents, les deux indices ont été appliqués par régression à des estimations de l'abondance de l'aiglefin d'un secteur différent, mais voisin, sur la partie sud-ouest du plateau Scotian. Des corrélations positives ont été observées avec les classes d'âge des immatures et les deux indices, alors qu'un seul des deux a donné une corrélation positive avec les classes d'âge des sujets matures. Les facteurs suivants ont contribué à alimenter la discordance entre les indices : 1) les indices appliqués à des populations agrégées par groupe d'âge peuvent masquer les corrélations entre l'abondance et surface occupée qui sont observées dans des classes d'âge individuelles; 2) les indices qui dépendent de l'importance du taux de prise mesurent à la fois la distribution horizontale à grande échelle des stocks et des variations diurnes dans la distribution des bancs de poissons en trois dimensions; 3) les indices qui varient dans le même sens que l'abondance sont à l'origine de fausses corrélations.

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Annual bottom trawl surveys are one of the principal tools used to monitor temporal variation in the abundance of groundfish populations (Doubleday and Rivard 1981). Abundance indices from trawl surveys are usually expressed as the mean number (or biomass) of each age-class caught per standard tow (Smith 1988). Recently, these data have been used to describe the distribution of groundfish stocks over a wide range of spatial and temporal scales. Mahon and Sandeman (1985) used survey data for the northwest Atlantic (Cape Hatteras to Hudson Strait) to delimit the occurrence of over 80 species of groundfish. Survey data have also been used to describe interannual (Pálsson and Stefánsson 1991; Atkinson 1992) and seasonal (Overholtz 1985) variation in the distribution of individual stocks. On finer spatial and temporal scales, diurnal variation in catch rates of cod and haddock has been observed during trawl surveys of the Barents Sea (Engås and Soldal 1992) and the continental shelf of the eastern United States (Shepherd and Forrester 1987), reflecting day/night differences in the vertical distribution and/or aggregation of fish schools (Beamish 1966; Boudreau 1992).

Studies that examine the effect of interannual variation in abundance on the large-scale horizontal distribution of marine fish stocks often assume that distributional area is directly proportional to simple, quantitative indices (Table 1). However, the strength of the correlations between these indices and estimates of abundance varies widely among studies and within stocks. For example, Murawski and Finn

TABLE 1. Summary of studies that examined the relationship between distributional area and abundance. SPA refers to an abundance index generated from sequential population analysis. CPUE is an abundance index derived from survey or commercial catch rates. The distributional indices are coded as follows: a, mean distance from the centre of distribution; b, Greens dispersion index; c, integrated area of strata where mean catch exceeds long-term median catch; d, frequency of zero catches; e, contoured area where catch-at-age exceeds the 60th percentile. Area refers to total area surveyed. In the case of Crecco and Overholtz (1990) and Lange (1991), this quantity was estimated from data in Grosslein (1974). Sets indicates the approximate number of stations sampled during each survey.

			Abundance		Distribution				
Species	Stock	Age	Source	Index	Index	Area (km^2)	Sets (no.)	r^2	Reference(s)
Silver hake	Georges Bank	1+	Surveys	CPUE	a	96 790	44–187	<0.01	Murawski and Finn 1988
		0			a			0.01	
		1+			b			0.34	
		0			b			0.01	
Atlantic cod	Georges Bank	1+	Surveys	CPUE	a	96 790	44–187	0.15	Murawski and Finn 1988
		0			a			0.09	
		1+			b			0.01	
		0			b			0.07	
Haddock	Georges Bank	1+	Surveys	CPUE	a	96 790	44–187	0.15	Murawski and Finn 1988
		0			a			0.07	
		1+			b			0.15	
		0			b			<0.01	
Pollock	Georges Bank	1+	Surveys	CPUE	a	96 790	44–187	0.08	Murawski and Finn 1988
		1+			b			0.20	
		0			b			0.27	
Red hake	Georges Bank	1+	Surveys	CPUE	a	96 790	44–187	0.20	Murawski and Finn 1988
		0			a			0.11	
		1+			b			0.01	
		0			b			0.09	
Yellowtail flounder	Georges Bank	1+	Surveys	CPUE	a	96 790	44–187	0.04	Murawski and Finn 1988
		0			a			0.19	
		1+			b			0.11	
		0			b			0.07	
Winter flounder	Georges Bank	1+	Surveys	CPUE	a	96 790	44–187	0.17	Murawski and Finn 1988
		0			a			0.11	
		1+			b			<0.01	
		0			b			0.22	
Haddock	Georges Bank	2+	SPA	Biomass	c	38 120	48	0.74	Crecco and Overholtz 1990
Longfin squid	Mid-Atlantic	All	Fishery	CPUE	d	95 040	111	0.54	Lange 1991
					b			0.07	
Atlantic cod	Gulf of St. Lawrence	3	SPA	Numbers	e	67 870	61–164	0.63	Swain and Wade 1993
		4			e			0.78	
		5			e			0.87	
		6			e			0.94	
		7			e			0.94	
		8+			e			0.90	

(1988) used several different indices to estimate the distributional area of Georges Bank haddock (*Melanogrammus aeglefinus*), including the mean distance of all sampling stations for which the catch-per-tow was greater than zero from a fixed reference point. They concluded that interannual variation in the distributional area was independent of abundance for both juvenile (0-group) and adult (age 1 and older) haddock. Crecco and Overholtz (1990) estimated the distributional area for Georges Bank haddock by integrating subareas for which the catch-per-tow exceeded the long-term median catch-per-tow. They observed a significant, positive correlation between distributional area and total abundance of haddock age 2 and older. The discrepancy between the conclusions of these two studies suggests that different indices measure different things, a difficulty in the analysis of spatial data noted previously by Pielou (1977) and Gaston (1991). Consequently, the assumption that all of the distributional indices represented in Table 1 are directly proportional to distributional area could be invalid.

The distributional indices represented in Table 1 can be categorized either as indices that discretize catch-per-tow data into zero and non-zero observations (e.g., the mean distance of all stations having catch-per-tow values that are greater than zero from a fixed reference point used by Murawski and Finn 1988) or as indices that depend on the magnitude of the catch-per-tow (e.g., the index used by Crecco and Overholtz 1990). There are potential problems with both types of indices. If catch-per-tow values vary systematically with time of day, indices that depend on the magnitude of the catch-per-tow will confound variation in the large-scale horizontal distribution of a stock with a diurnal signal in the small-scale three-dimensional distribution of fish schools. Many distributional indices scale positively with abundance (Gaston 1990); however, few studies inves-

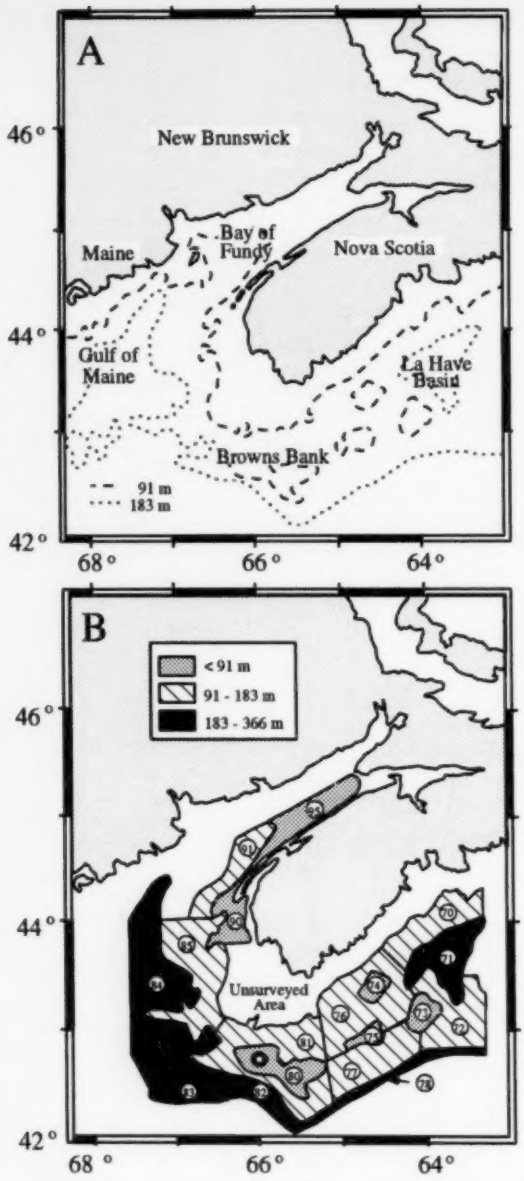

FIG. 1. Map of the southwestern Scotian Shelf. (A) Depth contours (91 and 183 m) used to define strata boundaries; (B) stratification scheme used for annual bottom trawl surveys. The star in Fig. 1B indicates the location used to calculate the mean distance from the centre of distribution.

tigate the potential spuriousness of positive correlations between distributional area and abundance. Finally, many of the distributional indices represented in Table 1 were estimated for the age-aggregated population. Since there is considerable evidence that the spatial distribution of groundfish varies with age (Overholtz 1985; Tremblay and Sinclair 1985; Pálsson and Stefánsson 1991; Wigley and Serchuk 1992; Swain and Wade 1993), age-aggregated indices potentially confound age effects with abundance effects.

The objectives of this study are (1) to determine whether the contradictory results obtained by Murawski and Finn (1988) and Crecco and Overholtz (1990) reflect the choice of distributional indices and are therefore reproducible for other haddock stocks and (2) to identify factors which contribute to the lack of agreement among distributional indices. Both the mean distance of all stations having catch-per-tow values greater than zero from a reference point corresponding to the long-term centre of distribution and the distributional index developed by Crecco and Overholtz (1990) were calculated for five age-classes (2, 3, 4, 5, and 6 yr old) of haddock on the southwestern Scotian Shelf using data from annual bottom trawl surveys. Both distributional indices were regressed against estimates of abundance-at-age and the results were compared with those obtained by Murawski and Finn (1988) and Crecco and Overholtz (1990). Scaled catch-at-age values and the frequency of non-zero catch-at-age values were plotted against the time of day when the sample was collected to assess whether the small-scale distribution of haddock varies diurnally. The likelihood that distributional indices scale positively with abundance, thereby generating spurious correlations, is also discussed.

Data Sources

Bottom Trawl Surveys

Bottom trawl surveys of the southwestern Scotian Shelf (NAFO Division 4X) have been conducted every summer since 1970 by the Canadian Department of Fisheries and Oceans (Halliday and Koeller 1981). The surveyed portion of NAFO Division 4X includes shallow offshore banks, a deep basin, and portions of the upper continental slope (Fig. 1A). The inshore area is unsurveyed due to an untrawlable bottom. The surveys use a stratified random design with depth as the major stratifying variable (Fig. 1B). Strata can be divided into shallow (<91 m), intermediate (91–183 m), and deep (183–366 m) representing 15, 55, and 30% of the total surveyed area (56 734 km^2), respectively. At each station, groundfish were captured with a Western IIA bottom trawl equipped with a 19-mm codend liner towed at a constant speed for approximately 30 min. Catch rates were adjusted to represent the abundance of fish in a standard tow of distance 3.25 km. Each trawl sample is subsequently referred to as a set. Sampling stations were located randomly and a minimum of two stations were sampled in each stratum. Additional stations were allocated approximately in proportion to stratum area. Sampling was conducted around the clock and, since there was no fixed schedule, the allocation of day and night sets varied randomly among strata and among years.

Abundance Indices

Two indices of abundance-at-age were used in the analysis: the mean catch-at-age for each survey and estimates of the total abundance-at-age obtained from sequential population analysis (SPA). The mean catch of age j haddock in year t ($\bar{y}_{jt}$) was estimated for t = 1970–90 by

$$(1)\quad \bar{y}_{jt} = \sum_{i=1}^{I} \frac{A_i}{A} \bar{y}_{ijt}$$

where A_i/A is the ratio of stratum area (A_i) to the total surveyed area (A). The arithmetic mean catch-at-age ($\bar{y}_{ijt}$) was estimated for each stratum by

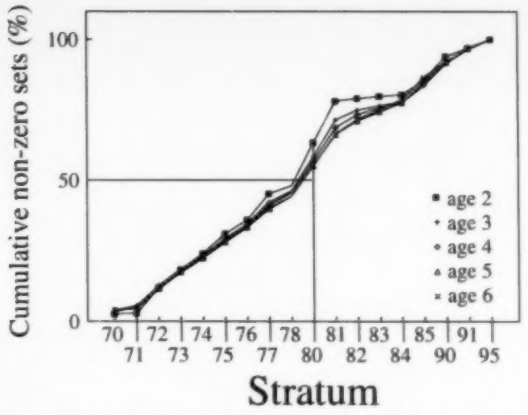

FIG. 2. Cumulative percentage of sets for which the catch-at-age of haddock age 2–6 was greater than zero by stratum.

$$\bar{y}_{ijt} = \frac{\sum_{h=1}^{H_i} y_{hijt}}{H_i}$$

where y_{hijt} is the total catch of age j haddock in set h of stratum i and H_i is the total number of sets in stratum i (Smith 1988). SPA estimates of total abundance-at-age were only available for 1970–88 (O'Boyle et al. 1989). Since the SPA was calibrated (sensu Rivard 1989) using survey estimates of the abundance of haddock age 2–8 as the independent variable, SPA estimates of abundance-at-age are not independent of the survey data.

Data Analysis

Murawski and Finn (1988) identified the centre of distribution for Georges Bank haddock by calculating the mean latitude and longitude of all sets weighted by the magnitude of the catch. This method was inappropriate for NAFO Division 4X haddock because it did not exclude the possibility that the centre would be located in the mainland of Nova Scotia or in the unsurveyed inshore area. Instead, all non-zero values of y_{hijt} were pooled (1970–90) and the location of the centre of distribution was determined by plotting the cumulative frequency of non-zero sets by strata. Browns Bank (stratum 80) was chosen as the centre of the distribution because the 50% mark in the cumulative distribution of non-zero sets was closest to this stratum for all five age-classes (Fig. 2). Therefore, the overall frequency of non-zero sets lying to the east of Browns Bank is comparable with the overall frequency of non-zero sets lying to the west of Browns Bank. Furthermore, all age-classes of haddock are very abundant on Browns Bank (Frank et al. 1990) and Browns Bank is the primary location of spawning in NAFO Division 4X (Waiwood and Buzeta 1989). The location of the centre of distribution (42.45°N, 66.02°W) is shown in Fig. 1B. The distance (kilometres) between all non-zero sets and the centre of distribution was determined using ACON version 5.02.[1] The arithmetic mean distance of all non-zero sets from the centre of distribution was then calculated for each survey.

TABLE 2. Area, number of sets, and total number and percentage of daytime sets by stratum for bottom trawl surveys (1970–90) conducted in NAFO Division 4X.

Stratum	Area (km^2)	Total sets (no.)	Daytime sets (no.)	Daytime sets (%)
70	3 160	43	26	60.5
71	3 448	42	29	69.0
72	4 289	54	35	64.8
73	910	43	36	83.7
74	553	40	27	67.5
75	536	42	23	54.7
76	5 076	52	31	59.6
77	4 231	53	37	69.8
78	800	55	34	61.8
80	2 249	80	61	76.3
81	6 439	93	59	63.4
82	3 579	47	32	68.1
83	1 827	41	29	70.7
84	7 775	64	43	67.2
85	5 433	69	50	72.5
90	2 064	61	40	65.6
91	2 359	64	43	67.2
95	2 006	41	26	63.4
Total	56 734	984	661	67.2

The distributional index developed by Crecco and Overholtz was calculated for each survey by summing the areas of strata where the mean catch-at-age ($\bar{y}_{ijt}$) exceeded the long-term median value of $\bar{y}_{ijt}$ (1970–90). The Crecco and Overholtz index was not calculated for the 1984 survey because stratum 74 was not sampled that year.

Since the total area surveyed is finite, the relationship between abundance-at-age and distributional area is likely to be nonlinear. Consequently, Murawski and Finn (1988) log-transformed indices of distributional area and abundance prior to fitting a model using simple linear regression. Crecco and Overholtz (1990) used nonlinear regression to estimate the parameters of a power equation relating distributional area and abundance. We chose to fit the logarithmic model

$$\text{Distribution area}_{jt} = a + b \cdot \log_e \text{Abundance}_{jt}$$

to the data using simple linear regression. This model was fit using both SPA and survey estimates of abundance-at-age. Visual inspection of the residuals did not reveal any consistent pattern associated with either age or year.

Diurnal Variation in Catch Rates

Sunrise/sunset times, estimated for Browns Bank, were 04:57/19:52 and 04:51/20:09 for the earliest and latest sampling date in the 21-yr time series, respectively (Atmospheric Environment Service, Environment Canada, Bedford, N.S.). Therefore, approximately 63% of the 24-h day was defined as daytime. Of the 984 stations sampled between 1970 and 1990, 661 (67%) were sampled during daylight. The allocation of daytime sets among strata varied between 54.7 and 83.7% (Table 2). The deviation from 63% was greatest for small strata (strata 73 and 75) due to the shorter distances between sampling stations. All non-zero values of y_{hijt} for surveys conducted between 1970 and 1990 were scaled by dividing by the mean catch-at-age for each survey ($\bar{y}_{jt}$) to remove interannual variation in abundance. The start

[1]G.A. Black. 1991. ACON - Documentation for ACON version 5.02. Unpublished manuscript, Department of Fisheries and Oceans. 148 p.

TABLE 3. Coefficient of determination and significance level (in parentheses) for models describing the relationship between the mean distance from the centre of distribution and SPA (n = 19) and survey (n = 21) estimates of abundance-at-age.

Age	SPA	Survey
2	0.54 (<0.001)	0.60 (<0.001)
3	0.46 (0.001)	0.36 (0.004)
4	0.04 (0.410)	0.09 (0.182)
5	0.01 (0.702)	<0.01 (0.704)
6	0.12 (0.140)	0.29 (0.012)

TABLE 4. Coefficient of determination and significance level (in parentheses) for models describing the relationship between the Crecco and Overholtz index and SPA (n = 18) and survey (n = 20) estimates of abundance-at-age.

Age	SPA	Survey
2	0.73 (<0.001)	0.63 (<0.001)
3	0.47 (0.002)	0.69 (<0.001)
4	0.54 (0.001)	0.62 (<0.001)
5	0.72 (<0.001)	0.79 (<0.001)
6	0.67 (<0.001)	0.80 (<0.001)

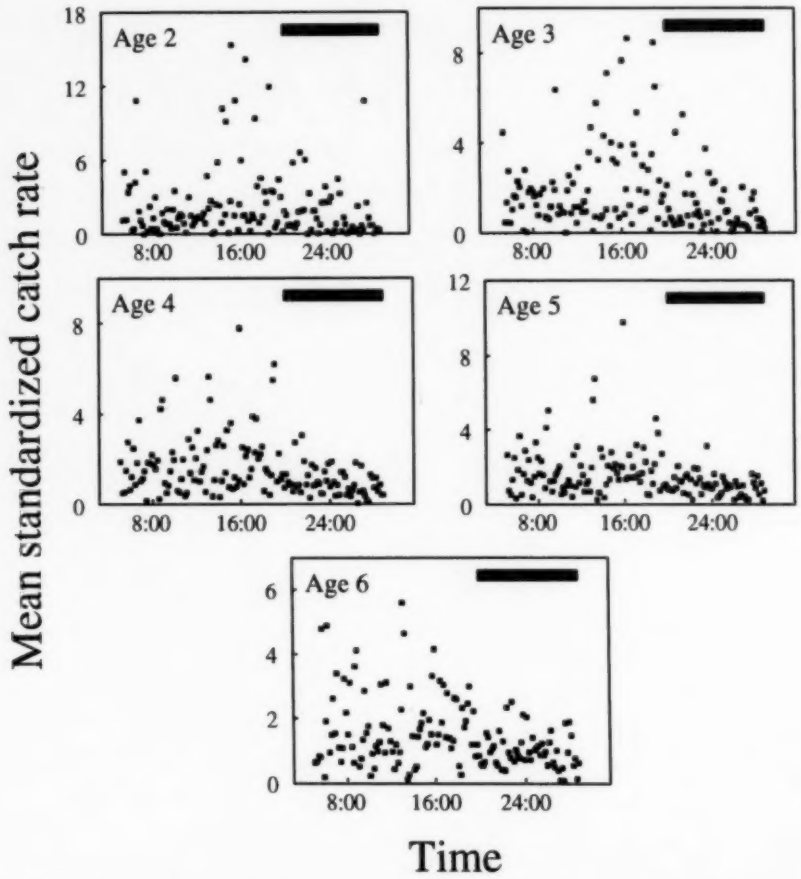

FIG. 3. Relationship between the standardized catch rate and time of day for haddock age 2–6. Each observation represents the mean standardized catch rate for each start time. The horizontal bar indicates the period between sunset (20:05) and sunrise (04:55).

time, rounded to the nearest 10-min mark, was recorded for each set. All scaled non-zero catch-at-age values recorded for each start time were averaged and plotted against time of day. Secondly, the mean daytime and the mean nighttime catch rates were calculated for each survey using only non-zero values of y_{hijr}. Lastly, the frequency of non-zero sets for each start time was plotted against time of day to determine whether the frequency of non-zero sets varied diurnally.

Results

Positive correlations ($p < 0.01$) between abundance-at-age and distributional area were observed only for haddock age 2 and 3 using the mean distance from Browns Bank to represent distributional area (Table 3). Abundance-at-age and distributional area were generally not correlated ($p > 0.05$) for haddock age 4, 5, and 6 using either the SPA or the

survey index of abundance. With the possible exception of age 6 haddock, there was little difference between the results obtained using the two abundance-at-age indices. Since Murawski and Finn (1988) used age-aggregated indices to represent the distributional area and abundance of Georges Bank haddock, the analysis was repeated using age-aggregated estimates of distributional area and abundance for haddock age 2–6. There was no significant correlation between distributional area and either SPA (p = 0.33) or survey (p = 0.44) estimates of abundance of haddock age 2–6. This result is consistent with results obtained by Murawski and Finn (Table 1) and suggests that age-aggregated indices obscure significant correlations between distributional area and abundance for individual age-classes of haddock.

Using the Crecco and Overholtz index to represent distributional area, positive correlations ($p < 0.01$) were observed for all five age-classes using either SPA or survey estimates of abundance-at-age (Table 4). This result is consistent with the results obtained by Crecco and Overholtz (1990), who observed a significant, positive correlation using an age-aggregated index to represent the distributional area of adult haddock on Georges Bank. It is also consistent with the results of Swain and Wade (1993), who used an age-specific index similar to that developed by Crecco and Overholtz to represent the distributional area of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence (Table 1). However, the significant correlations for haddock age 4, 5, and 6 contradict the results obtained using the mean distance from Browns Bank to represent the distributional area. The lack of agreement for older haddock on the southwestern Scotian Shelf suggests that the two distributional indices do not represent the same thing.

Diurnal Variation

Non-zero catch rates are lognormally distributed (Pennington 1983); therefore, it is not surprising that the overall frequency of high catch rates is low relative to the overall frequency of low catch rates (Fig. 3). High catch rates were more frequent during the day for all five age-classes of haddock, particularly for haddock age 3–6. High catch rates were rarely observed at night and maximum catch rates were most frequently observed in the 4 h preceding sunset. A similar pattern was observed when the data set was divided into two parts: the two strata having consistently high catch rates (strata 80 and 90) and the remaining 16 strata. For age 2 haddock, mean nighttime catch rates were not consistently higher or lower than mean daytime catch rates (Fig. 4A). However, mean daytime catch rates for haddock age 3–6 were often more than double the mean nighttime catch rates (Fig. 4B). No systematic pattern of variation was observed in plots of the frequency of non-zero sets versus start time for haddock age 2–6 (Fig. 5). For haddock on the southwestern Scotian Shelf, treating the survey data as dichotomous data (zero and non-zero values) eliminates the potential for variation in the small-scale distribution of schools to confound the analysis of the large-scale distribution of a stock.

Discussion

Both distributional indices showed significant, positive correlations between distributional area and abundance-at-age

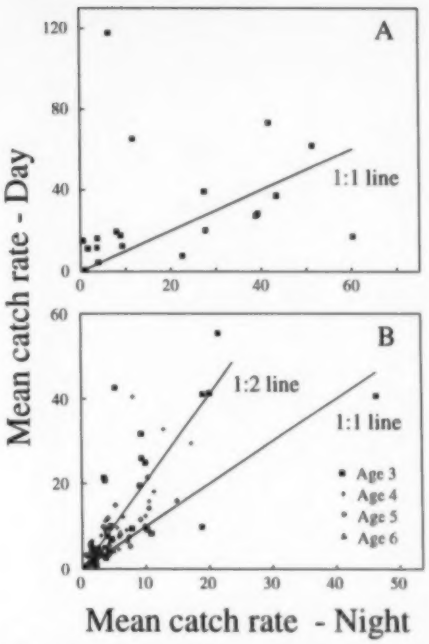

FIG. 4. Relationship between the mean nighttime and mean daytime catch rates calculated using non-zero observations only. Each observation represents a single survey. (A) Data for age 2 haddock; (B) data for age 3, 4, 5, and 6 haddock. The 1:1 and 1:2 lines are also indicated.

for haddock age 2–3. The majority of haddock in these two age-classes are immature (Waiwood and Buzeta 1989) and tagging studies on the Scotian Shelf indicated that immature haddock are more sedentary and less widely distributed than mature haddock (Needler 1930). This may make their distributional response to variation in abundance easier to detect using data having a low degree of spatial resolution. The two distributional indices gave contradictory results for haddock age 4, 5, and 6; distributional area and abundance-at-age were uncorrelated using the mean distance from Browns Bank as a distributional index whereas the Crecco and Overholtz index gave positive correlations. This inconsistency is analagous to that observed for haddock on Georges Bank (Table 1) and implies that at least one of the two indices is not directly proportional to distributional area. Since it is not possible to estimate distributional area directly, identifying which index is more likely to be proportional to the "true" distributional area requires that potential sources of error be identified and their impact on the correlation analysis assessed.

The mean distance of all non-zero sets from the centre of distribution depends on geographic information rather than the magnitude of catch rates. Like most distributional indices, this index is sensitive to differences among surveys in the spatial distribution of sampling effort. For example, a disproportionate increase in the number of samples collected in stratum 80 would decrease the value of the mean distance from the centre of distribution because haddock are always caught in this stratum. Although the total number of stations sampled has increased be approximately 33% since 1987 (Table 5), the allocation of sampling effort

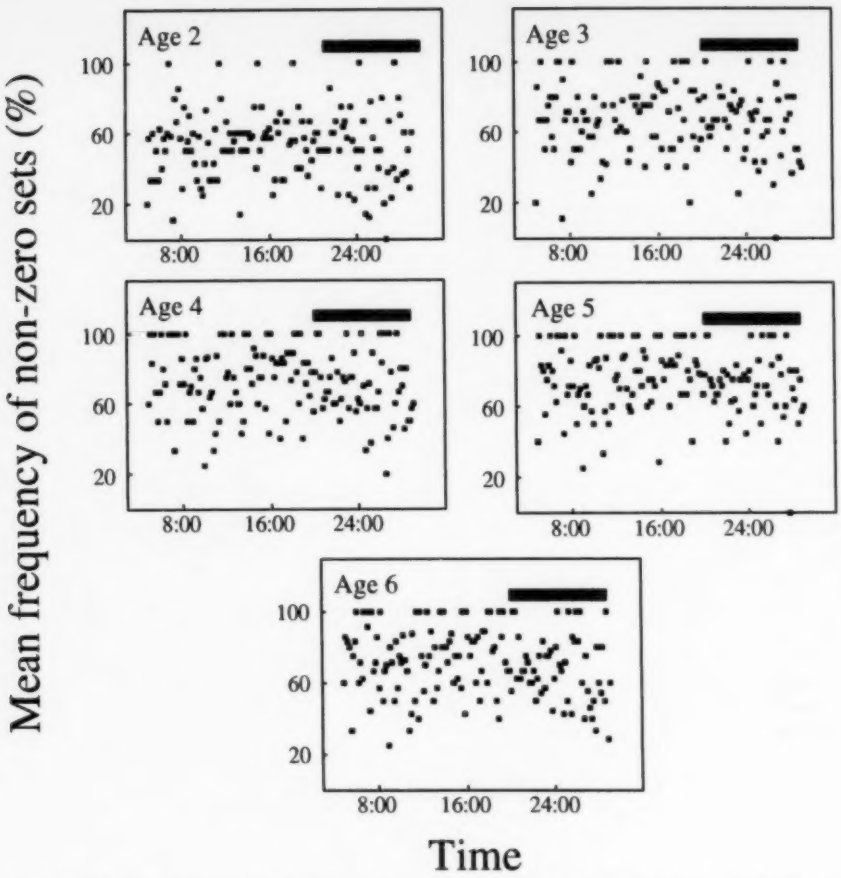

FIG. 5. Relationship between the frequency of non-zero sets and time of day for haddock age 2–6. Each observation represents the frequency of non-zero sets for each start time. The horizontal bar indicates the period between sunset (20:05) and sunrise (04:55).

among strata has been relatively constant. By definition, the mean distance of all non-zero sets from the centre of distribution is also dependent on the location of the centre. Although the assumption that Browns Bank is the centre of distribution is justifiable for haddock, it is more difficult to identify centres of distribution for stocks composed of discrete subunits (e.g., Hunt and Neilson (1993) have suggested that the Division 4X cod "stock" is composed of at least two distinct subunits) or for stocks that shift their distribution over time (e.g., Mohn and MacEachern (1992) have suggested that the distribution of cod on the eastern Scotian Shelf has shifted to deeper water).

Distributional indices that depend on the magnitude of the catch rates potentially confound variation in the large-scale horizontal distribution of haddock stocks with variation in the small-scale vertical distribution of schools. High catch rates of haddock on the southwestern Scotian Shelf are more frequent during the daytime. This is consistent with acoustic data which indicate that during the day, schools of haddock on Browns Bank contract to form dense aggregations whereas at night, their distribution is more diffuse and densities are lower (Boudreau 1992). Video and photographic observations show day/night differences in both the density and swimming behaviour of Scotian Shelf haddock near the trawl opening (Walsh and Hickey 1993). Similarly, spatial variation in hydrographic variables (e.g., temperature, salinity) is known to influence catch rates of haddock (Murawski and Finn 1988; Polacheck and Vølstad 1993) and may also affect the value of distributional indices.

Although hydrographic and/or diurnal variation in catch rates may decrease the precision of distributional indices that depend on the magnitude of catch rates, it is not responsible for the positive correlations between distributional area and abundance that are *consistently* observed using the Crecco and Overholtz index. Assuming that catch rates have a negative binomial distribution, Murawski and Finn (1988) demonstrated that the probability of catch rates exceeding zero increased as mean abundance increased. They concluded that using the frequency of zero (or non-zero) sets as an index of the large-scale horizontal distribution of fish stocks (e.g., Bannerot and Austin 1983; Lange 1991) was invalid. Since the probability of catch rates exceeding a constant value (e.g., the long-term median catch-per-tow) will also increase with increasing abundance, the distributional index developed by Crecco and Overholtz (1990) scales positively with abundance. Since the positive correlations

TABLE 5. Sampling dates and total number of sets for bottom trawl surveys of NAFO Division 4X.

Year	Start date	End date	Sets (no.)
1970	07/07	07/16	40
1971	06/30	07/08	38
1972	06/25	07/01	43
1973	07/10	07/19	42
1974	07/09	07/23	44
1975	07/15	07/24	44
1976	07/13	07/19	45
1977	07/10	07/16	45
1978	07/10	07/13	42
1979	07/06	07/13	45
1980	07/07	07/14	42
1981	07/06	07/12	44
1982	07/10	07/18	47
1983	07/07	07/14	45
1984	07/10	07/20	43
1985	07/04	07/10	45
1986	07/08	07/14	45
1987	06/29	07/09	60
1988	07/04	07/11	62
1989	07/05	07/13	60
1990	07/04	07/12	63

observed for age 4, 5, and 6 haddock using the Crecco and Overholtz index (Table 4) were not corroborated by a distributional index based on geographic information alone (Table 3), these correlations should be considered spurious. The results of studies that have used the Crecco and Overholtz index and found positive correlations between distributional area and abundance (e.g., Gordoa and Hightower 1991) should be confirmed using alternative methods of describing the distributional response to varying abundance.

It is obvious from the preceding discussion that most, if not all, distributional indices used to summarize data from bottom trawl surveys are subject to criticism. A more comprehensive approach would combine correlation analysis (e.g., Table 1) with a description of regional differences in the *rate* at which groundfish increase their local abundance in response to increases in total abundance. For example, Swain and Wade (1993) detected significant differences among subareas in the slope of the relationship between log-transformed catch rates and total abundance-at-age for cod in the Gulf of St. Lawrence. Swain and Wade concluded that as total abundance increased, local abundance increased slowly in subareas where cod were abundant and more rapidly in surrounding subareas. To examine regional differences in the rate at which age 2 haddock in NAFO Divison 4X increase their local abundance, the frequency of non-zero sets was plotted against total abundance-at-age for three subareas (Fig. 6). The frequency of non-zero sets in Browns Bank was 100% in 15 of 17 yr, which is consistent with Browns Bank being the centre of distribution. As total abundance increases, the frequency of non-zero sets increased more rapidly on the offshore banks lying to the east of Browns Bank (strata 73–77) relative to strata in the Bay of Fundy (strata 85, 90, 91, and 95). Thus, age 2 haddock expand and contract their distribution around Browns Bank in response to increases and decreases in abundance, respectively. Regional differences in the rate at which haddock increase their local abundance in response to increasing total abundance could be compared with other variables (e.g., growth rate, temperature, food availability) to identify spatial gradients in habitat suitability.

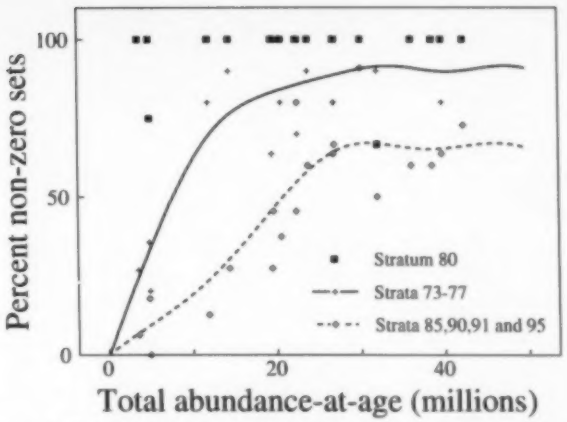

FIG. 6. Relationship between the frequency of non-zero sets for age 2 haddock and total abundance-at-age for Browns Bank and two subareas of NAFO Division 4X. The smoothed lines were calculated by distance-weighted least squares. The total area and sampling effort are approximately equal for strata 73–77 and strata 85, 90, 91, and 95 (Table 2).

Conclusions

In the ecological literature, the interaction between the abundance and distribution of populations has been exclusively described using cross-species comparisons (Gaston 1990). Bottom trawl surveys offer a unique opportunity to describe the distributional response of individual populations to variation in abundance over broad spatial and temporal scales. The challenge is to develop appropriate quantitative methods for analyzing data having an inherently low degree of spatial resolution. We have stressed the importance of critically evaluating distributional indices that are estimated from survey data because poorly defined indices lead to the proliferation of conflicting results. To reduce the chance of erroneous conclusions caused by spurious correlations, correlational approaches could be combined with more detailed analysis of the covariation between local and total abundance for individual stocks (e.g., Myers and Stokes 1989; Swain and Wade 1993).

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Accuracy and Choice in Risk Estimation for Fisheries Assessment

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Cordue, P.L., and R.I.C.C. Francis. 1994. Accuracy and choice in risk estimation for fisheries assessment. Can. J. Fish. Aquat. Sci. 51: 817–829.

Risk analysis has been used recently to enhance scientific advice to managers by providing estimates of risk to the fishery of different management strategies. However, little consideration has been given to the accuracy of these estimates. We present a reformulation and generalization of the risk analysis procedure of Francis (1992. Can. J. Fish. Aquat. Sci. 49: 922–930) and use simulation methods to examine the properties of a number of alternative risk estimators for two of New Zealand's main fisheries. It is shown that the choice of estimator can strongly affect the final estimates of risk and that the risk estimators can be alarmingly inaccurate. The accuracy of estimates is also shown to vary according to the type of risk being estimated, so analysts may improve the accuracy of their estimates by choosing the type of risk they estimate.

On a récemment eu recours à l'analyse de risque pour améliorer la qualité des conseils scientifiques fournis aux gestionnaires en fournissant des estimations des risques pour les pêches, de différentes stratégies de gestion. Toutefois, on s'est assez peu soucié de l'exactitude de ces estimations. Nous présentons ici une reformulation et une généralisation de la procédure d'analyse de risque de Francis (1992. J. can. sci. halieut. aquat. Sc. 49 : 922–930) et nous avons recours à des méthodes de simulation pour examiner les propriétés de différents estimateurs de risque développées pour deux des principales pêches de la Nouvelle-Zélande. Il est prouvé que le choix d'un estimateur peut agir considérablement sur les estimations finales des risques et que les estimateurs de risque peuvent être dangereusement inexacts. Il est aussi montré que l'exactitude des estimations varie selon le type de risque à estimer; ainsi les analystes peuvent-ils améliorer l'exactitude de leurs estimations en choisissant attentivement le type de risque qu'ils veulent estimer.

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Risk analysis has been applied in (at least) two ways in fisheries science. The first approach concentrates on the long term and involves evaluating different harvesting policies (e.g., constant fishing mortality versus constant escapement) for a fishery (Mendelssohn 1979; Hall et al. 1988). The second approach is concerned primarily with the short term and provides a means for choosing between several short-term management strategies (Linder et al. 1987; Mohn 1991; Cordue et al. 1992; Francis 1992; Restrepo et al. 1992). In this second type of application (which is the subject of this paper) the intention is to provide better information to fishery managers by expressing uncertainty about the effects of the various management strategies in terms of "biological risk" (e.g., the probability of the spawning stock biomass falling below 20% of the virgin value within 5 yr).

In this paper, we focus on two questions that arose from specific applications of risk analysis in New Zealand stock assessments in 1991 (Francis and Robertson 1991; Cordue et al. 1992). These questions concern accuracy and choice. First, how accurately were the risks estimated? Second, how should we choose between the three methods of risk analysis that were used: the original (confidence distribution) method (Francis and Robertson 1990) and Bayesian and maximum likelihood variants of it (Cordue et al. 1992)?

To help in addressing these questions, we first present a reformulation of the original risk analysis procedure of Francis (1992). We define a family of risk estimators, which we call *D*-estimators, and show that the confidence distribution, Bayesian, and maximum likelihood procedures are all *D*-estimators. We also describe another *D*-estimator, the bootstrap, and extend our investigations to include it. The reformulation will be seen to substantially simplify the calculations involved in considering the questions of accuracy and choice. It will also aid our understanding of why there are differences in performance between two estimators and also between the results of the application of one estimator to two different fisheries.

We evaluate these risk estimators for the two fish stocks to which risk analysis was applied in the 1991 New Zealand stock assessments. These were the Chatham Rise stock of orange roughy, *Hoplostethus atlanticus* (Francis and Robertson 1991), and the western stock of hoki, *Macruronus novaezelandiae* (Cordue et al. 1992). We will show that for these two assessments, our estimates of risk were alarmingly inaccurate, so that although the main conclusions of the assessments are unchanged, our confidence in these conclusions is reduced. We also show that analysts can increase accuracy by choosing an appropriate type of risk. These results have implications for other applications of risk analysis.

Terminology

Three terms "probability", "risk", and "management strategy" need defining. Probability may be understood in terms of relative frequencies, or as an expression of degree of

belief. The first interpretation is that of frequentist, or classical, statistics; the second is the subjectivist interpretation (Regazzini 1988). We use both interpretations, following Francis (1992) (as clarified by Francis (1993). We see these interpretations as complementary, rather than mutually exclusive.

There are two common interpretations of the word "risk". We follow Francis (1991) by defining risk as the probability of a specified (adverse) event occurring within a given future time frame. This interpretation is widespread in the fisheries literature (Breiwick et al. 1981; Peterson and Smith 1982; Fletcher 1985; Brown and Patil 1986; Bergh and Butterworth 1987; Linder et al. 1987; Swartzman et al. 1987; Hall et al. 1988; Punt and Butterworth 1991; Francis 1992; Restrepo et al. 1992), although many authors do not explicitly define risk. It is also used in the wider scientific literature (e.g., Wilson and Crouch 1987). A second interpretation sees risk as "a compound measure comprising a probability of occurrence of an (adverse) event and a magnitude of the consequences" (Gough 1990). This is the interpretation used in decision theory (Ferguson 1967; Berger 1985). (It may be shown (details available from authors) that what we define below as "risk functions" would, in decision theory terminology, be called loss functions, and our "D-estimators of risk" are generalisations of Bayes expected loss.)

Risk analysis is concerned with estimating the risk to a fish stock of various management strategies. In this context, a management strategy is either a sequence of future catch levels (covering the time frame of our risk definition) or some rule for calculating such a sequence (e.g., a target fishing mortality).

Risk Analysis

Original Approach

In this section, we describe the original approach to risk analysis, as described by Francis (1992), with variants given by Cordue et al. (1992). We provide only sufficient detail for the purposes of the present paper; more details are given in the papers just cited.

For the risk analysis, two types of data are available. The first type, a complete catch history and biological parameters (natural mortality, growth, etc.), is considered to be known exactly. The second type, a set of biomass indices, I, in one or more time series, is considered to be observed with error. The coefficients of variation (c.v.'s) of the indices in I are assumed known. In simulating a fish stock from its virgin state up to the present, recruitment is treated as deterministic (calculated from a stock–recruitment relationship), and the virgin state of the stock is completely determined by the virgin biomass, B_0. This has two consequences. First, it means that the "present" status of the stock (i.e, the status at the end of the time period covered by the catch history) is determined completely by B_0. Second, there is a minimum possible value of B_0, which we will write as $B_{\min}$. This is the smallest value of B_0 that would allow the historical catches to have been caught.

In general, I will be the union of m independent time series of biomass indices (e.g., a combination of trawl indices, c.p.u.e. indices, and acoustic indices). The members of each time series are measures of relative, rather than absolute, abundance, and the "scaling" constants (q_i; $i = 1,\ldots,m$) associated with each series are unknown. Thus, the list of unknown parameters is made up of the unknown scaling parameters, q_i, and the unknown virgin biomass, B_0.

Stage 1 of the risk analysis involves determining which are the likely values of B_0, given the observed biomass indices, I. Specifically, we calculate a probability distribution for B_0, which we denote $D(I)$. Note that the q_i are "nuisance" parameters here in the sense that although I depends on both B_0 and the q_i, we wish to calculate a probability distribution for B_0 alone, given I. We consider four alternative ways of calculating $D(I)$: confidence distribution, Bayesian, bootstrap, and maximum likelihood. Details of these methods are given in the Appendix.

In stage 2 of the risk analysis, the fishery is simulated n times. On each run, a random B_0 is chosen from $D(I)$ and used, with the population model, to simulate the fishery up to the present (with deterministic recruitment). The fishery is then simulated into the future using stochastic recruitment and the future catch levels defined by the management strategy, and the occurrence of the adverse event (used to define risk) is tested for. The estimate of risk is the proportion of times that the event occurs over the n runs.

To express this estimation process formally, we define a random variable Y which takes value 1 if the adverse event occurs and 0 if it does not. The value of Y for any particular run will depend on the chosen value of B_0, the management strategy s, and the vector Z of normal random variates that determine the future (stochastic) recruitment. If we denote our estimate of risk for a given D and s as $R_s(D(I))$, then it is given by

$$R_s(D(I)) = E_{D(I),F}[\mathrm{Y}(B_0,s,Z)]$$

where F is the probability distribution for the normal random vector Z and the subscripts $D(I)$,F indicate that the expectation on the right-hand side is taken by integrating over B_0 (using the distribution $D(I)$) and Z (using F).

Strictly speaking, the equality in the last equation is only approximate because the expectation is calculated by numerical integration (in the simulation process of stage 2). However, we can make the error in this approximation as small as we like (by using a large enough n) and, as will be shown below, this error is small compared with the error arising from uncertainty in our knowledge of $D(I)$.

Reformulation of Risk Analysis

A different way of estimating risk involves splitting stage 2 of the previous section into two parts, 2A and 2B. This split will allow a better understanding of the whole process and simplify the simulations described in the next section. It will also allow us to define rigorously the unknown risk which is being estimated.

Define the risk function r_s by

$$\forall\ B_0 \geq B_{\min}$$

$$r_s(B_0) = E_F[\mathrm{Y}(B_0,s,Z)].$$

Then the risk we are trying to estimate (the risk associated with management strategy s) is $r_s(B_{0\mathrm{true}})$ where $B_{0\mathrm{true}}$ is the true (unknown) virgin biomass. (Note that an explicit definition of the quantity to be estimated is missing from earlier descriptions of this type of risk analysis. Also note that our risk estimate $R_s(D(I))$ is related to the risk function r_s by the equations

TABLE 1. Assumed c.v.'s of the biomass indices for hoki and orange roughy. The c.v.'s are given only for those years in which indices were available.

	1984	1985	1986	1987	1988	1989	1990
Hoki							
c.p.u.e.			0.43	0.35	0.35	0.37	0.35
Acoustics					0.33	0.25	0.23
Orange roughy							
Trawl	0.15	0.15	0.15	0.15	0.15	0.15	0.15

$$R_s(D(I)) = E_{D(I),F}[\Upsilon(B_0,s,Z)] = E_{D(I)}[E_F[\Upsilon(B_0,s,Z)]] = E_{D(I)}[r_s(B_0)].$$

Our reformulation of risk analysis replaces stage 2 (the calculation of the expectation $E_{D(I),F}[\Upsilon(B_0,s,Z)]$ by simulation) by stage 2A (estimation of r_s, described below), followed by stage 2B (the evaluation of $E_{D(I)}[r_s(B_0)]$ by simple numerical integration).

Thus, our new approach has shown that we may think of our risk estimate $R_s(D(I))$ as being the expected value of $r_s(B_0)$ where B_0 is a random variable with distribution $D(I)$. It also shows us that the confidence distribution, Bayesian, bootstrap, and maximum likelihood estimators are members of a family of risk estimators which we will call D-estimators. All members of this family satisfy the equations preceding this paragraph; they differ only in the definition of the function D.

A key point to note is that the risk function r_s is independent of D. That is, its evaluation in stage 2A is independent of the procedure(s) used (e.g., confidence distribution, Bayesian, or other) to evaluate D in stage 1. This will be of particular importance when we wish to evaluate $R_s(D(I))$ for fixed values of s and I and several different functions D.

The steps involved in stage 2A of our new approach (estimating the risk function r_s) are as follows: (i) choose a value of B_0, (ii) simulate the fishery n times starting with virgin biomass B_0 and using the observed catch history (and deterministic recruitment) up to the present, and the catches determined by s (and stochastic recruitment) in the future, (iii) estimate $r_s(B_0)$ as the average value of Y over the n runs, (iv) repeat steps (i) – (iii) for a range of values of B_0, and (v) fit a smooth curve through the estimated values of $r_s(B_0)$.

Evaluation of Five *D*-Estimators for Two Fisheries

The remainder of this paper is devoted to evaluating, by simulation, the performance of five different D-estimators of risk: the confidence distribution, maximum likelihood, and bootstrap estimators, and the two Bayesian estimators corresponding to two different Bayesian priors. The biological parameters, catch histories, biomass indices, risk definitions, and management strategies used in the simulations come from 1991 stock assessments for hoki (Cordue et al. 1992) and orange roughy (Francis and Robertson 1991).

The simulation methods described in the next section are complex. The following description may help in understanding them. Let us restrict attention to a single fishery, a single type of risk, a single D-estimator of risk, and a single management strategy. Suppose we know the true value of B_0, which we write as $B_{0\text{true}}$. Then we may calculate the true risk associated with the management strategy s, i.e., $r_s(B_{0\text{true}})$. However, the actual estimate of risk, $E_{D(I)}[r_s(B_0)]$ that would be calculated (without knowledge of $B_{0\text{true}}$) depends on the biomass indices I that are observed. The idea is to simulate 200 different sets of biomass indices that could have been observed had the true virgin biomass been $B_{0\text{true}}$. For each set of indices, we get an estimate of risk. How close these 200 estimates of risk are to the true risk, $r_s(B_{0\text{true}})$, is a measure of the performance of the D-estimator for that $B_{0\text{true}}$. We can then repeat the calculations for a range of $B_{0\text{true}}$ values and for different management strategies, different types of risk, different D-estimators, and different fisheries.

Simulation Methods

The procedure for a given fishery, a given type of risk, and a given B_0 was as follows: (i) run the population model with the true virgin biomass B_0 to obtain the true biomass in each year for which an index exists in I (the given set of biomass indices), (ii) simulate a set of biomass indices J (with the same years represented and containing the same number of indices as I) using the biomasses calculated in step (i) and the known c.v.'s of the indices in I, (iii) repeat steps (a) and (b) for each D-estimator: (a) derive the B_0 distribution $D(J)$ and (b) for each management strategy s, calculate and store $R_s(D(J))$, and (iv) repeat steps (ii) and (iii) 200 times.

In the simulations the catch levels specified by the management strategy were treated as targets; the actual catch in any year for a particular simulation run was the lesser of the target value and the value obtained by applying what is considered to be the maximum possible fishing mortality for that fishery.

Two Bayesian Priors

The Bayesian prior must be a joint distribution on the q_i and B_0. We assume that in the prior distribution, B_0 and the q_i are independent, so that the prior distribution for the full set of unknown parameters is the product of the priors for the individual parameters. Two different combinations of prior distributions have been considered in this paper:

$$B_0 \sim U[B_{\min},B_{\max}] \text{ and } \forall i\ q_i \sim U(0,\infty)$$

and

$$B_0 \sim U[B_{\min},B_{\max}] \text{ and } \forall i\ \log(q_i) \sim U(-\infty,\infty))$$

where $B_{\min}$ is the smallest value of B_0 which would have allowed the historical catches to have been caught, $B_{\max}$ is a subjective estimate of the maximum possible size of B_0, and $U[a,b]$ denotes a uniform distribution on the range $[a,b]$.

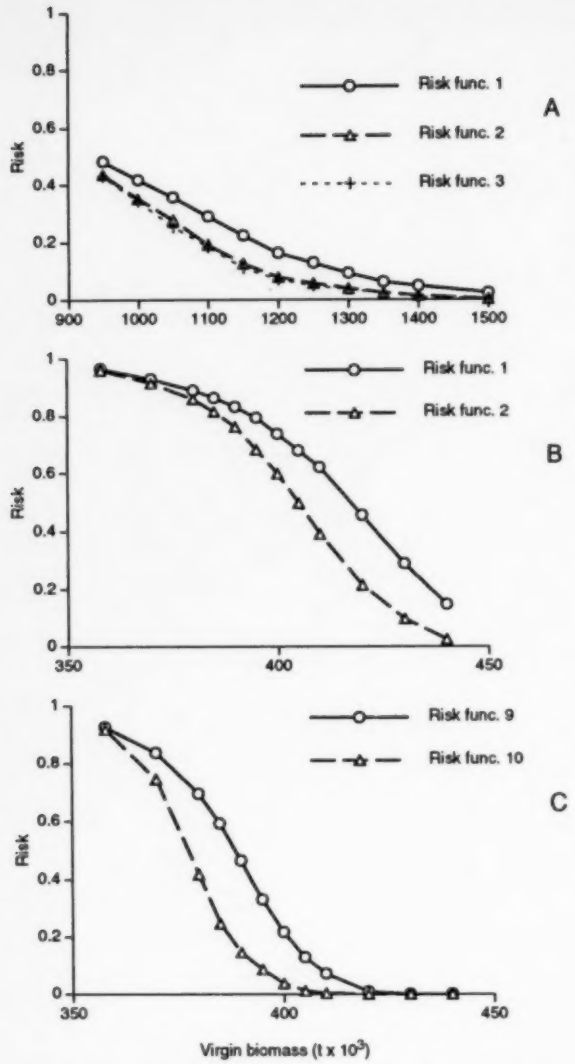

FIG. 1. Risk functions for the probability of (A) the midseason biomass falling below 20% of B_0 in the hoki fishery, (B) the midseason biomass falling below 10% of B_0 in the orange roughy fishery, and (C) the instantaneous fishing mortality reaching the maximum possible value of 1 in the orange roughy fishery. All probabilities are calculated for the period 1991–96, inclusive. The risk functions for hoki correspond to the three management strategies described for hoki in the section entitled "Fisheries"; those for orange roughy correspond to the two orange roughy management strategies in the same section.

These priors have been considered because they were the ones used by Cordue et al. (1992). Also, they are a recognized standard when analysts have little prior knowledge of the values of the parameters (Pack 1986). The question of what is the "best" prior to use is not considered here.

Fisheries

The time series of orange roughy indices is superior to both time series available for hoki in that it is longer and has lower c.v.'s (Table 1). Three management strategies were examined for hoki and two for orange roughy. The catch levels (thousands of metric tons (t)) for 1991 to 1996 for these strategies were as follows:

	1991	1992	1993	1994	1995	1996
Hoki						
(i)	150	150	150	150	150	150
(ii)	150	140	130	120	120	120
(iii)	150	140	130	120	110	110
Orange roughy						
(i)	23.8	18.8	13.8	8.8	7.5	7.5
(ii)	23.8	14.8	7.5	7.5	7.5	7.5

In the case of hoki, the three management strategies were examined under a single type of risk: the probability of the midseason biomass dropping below 20% of B_0 in any year from 1991 to 1996 inclusive. For orange roughy, two types of risk were used for each management strategy: as for hoki, but with a threshold of 10% of B_0, and the probability of the instantaneous fishing mortality being greater than 1 (assumed to be the maximum possible value for this fishery) in any year from 1991 to 1996 inclusive.

The simulations to derive risk estimates were performed, for each species, at B_0 values ranging from B_{min} to B_{max}: from 950 000 to 3 000 000 t for hoki and from 358 000 to 600 000 t for orange roughy. Further details of each fishery and their catch histories and biological parameters used may be found in Francis and Robertson (1991) and Cordue et al. (1992).

Results

There was a marked contrast between the risk for the two fisheries (Fig. 1). The maximum risks were smaller for hoki (0.43–0.48) than for orange roughy (0.92–0.96). Also, the difference between low-risk and high-risk values of B_0 was much less, in percentage terms, for orange roughy than for hoki. For example, B_0 values associated with a risk of 0.1 were only 10–26% higher than B_{min} (where risk is maximum) for orange roughy but were 22–36% higher for hoki.

These differences imply that the precise estimation of risk should be more difficult for orange roughy than for hoki because much more precise knowledge of B_0 would be required. Similarly, because the (orange roughy) risk functions for the B_0-threshold type of risk (Fig. 1B) are less steep than those for the fishing-mortality-threshold type (Fig. 1C), estimates of the former type of risk ought to be more precise.

For a given fishery, risk function, value of B_0, and D-estimator, 200 simulated risk estimates were generated. This set of estimates was used to approximate the probability density function (p.d.f.) for that D-estimator applied to the given fishery and risk function when the true virgin biomass is B_0. Examination of a number of p.d.f.'s, at different values of B_0, for particular risk functions indicates that the estimators are very inaccurate: their p.d.f.'s are not centred on the true risks and have a large degree of spread (Fig. 2 and 3).

The properties of the estimators differed somewhat between fisheries but were consistent, within a fishery, across management strategies or types of risk (for orange roughy). Therefore, we have generally restricted our presentation of

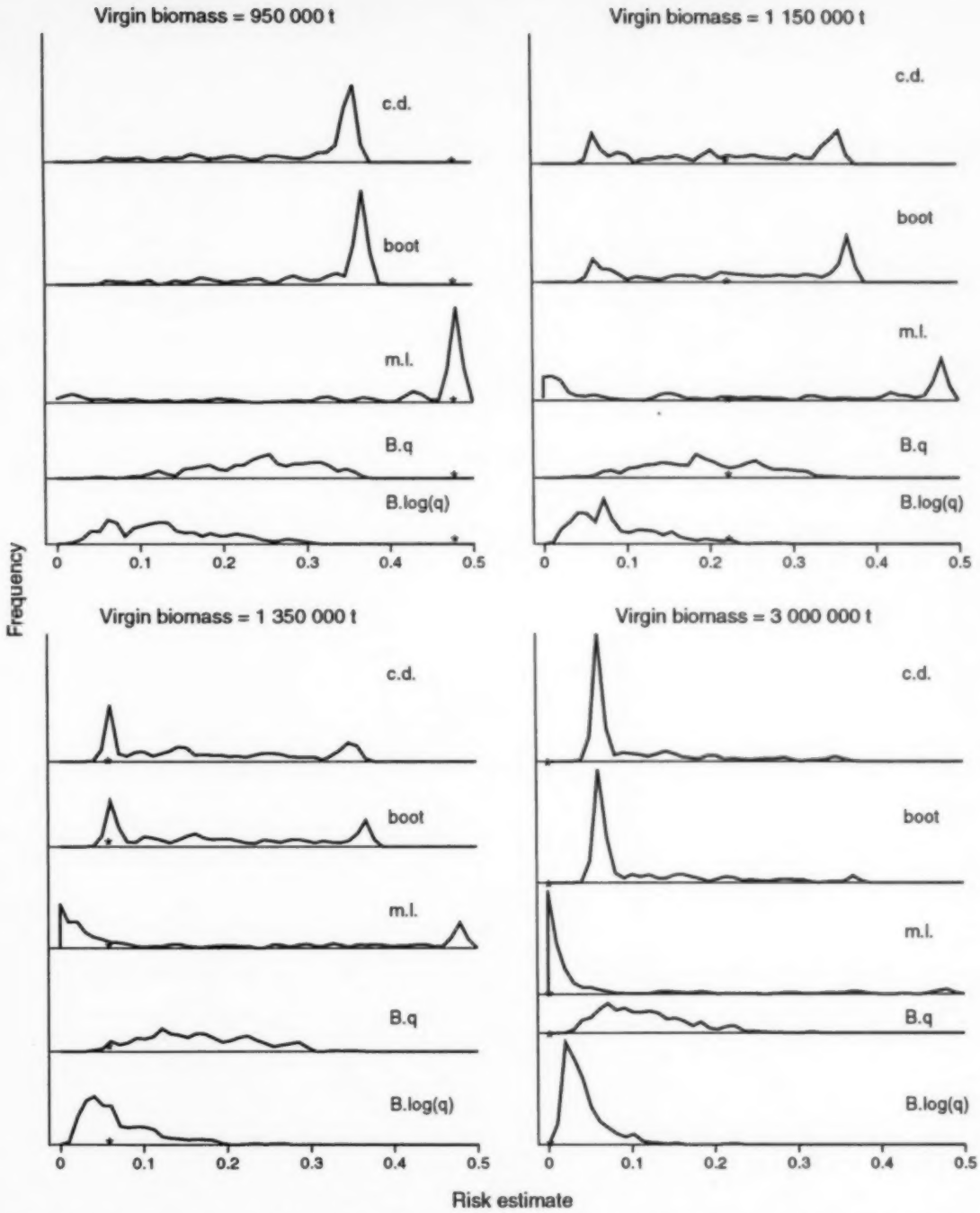

FIG. 2. Simulation results for hoki risk function 1. The approximate p.d.f.'s of each risk estimator are shown for given levels of virgin biomass, B_0. The true level of risk, for each level of virgin biomass, is shown by an asterisk.

results to a single risk function for each fishery: hoki risk function 1 and orange roughy risk function 9.

The performance of each estimator may be judged by the relationship between the estimator's p.d.f. and the true risk (i.e., $r_s(B_0)$, shown as asterisks in Fig. 2 and 3). We considered three measures of performance: bias, mean squared error, and the ability to differentiate between high and low risk.

In terms of bias, four conclusions can be drawn (Fig. 4). First, all the estimators are badly biased, tending to over-

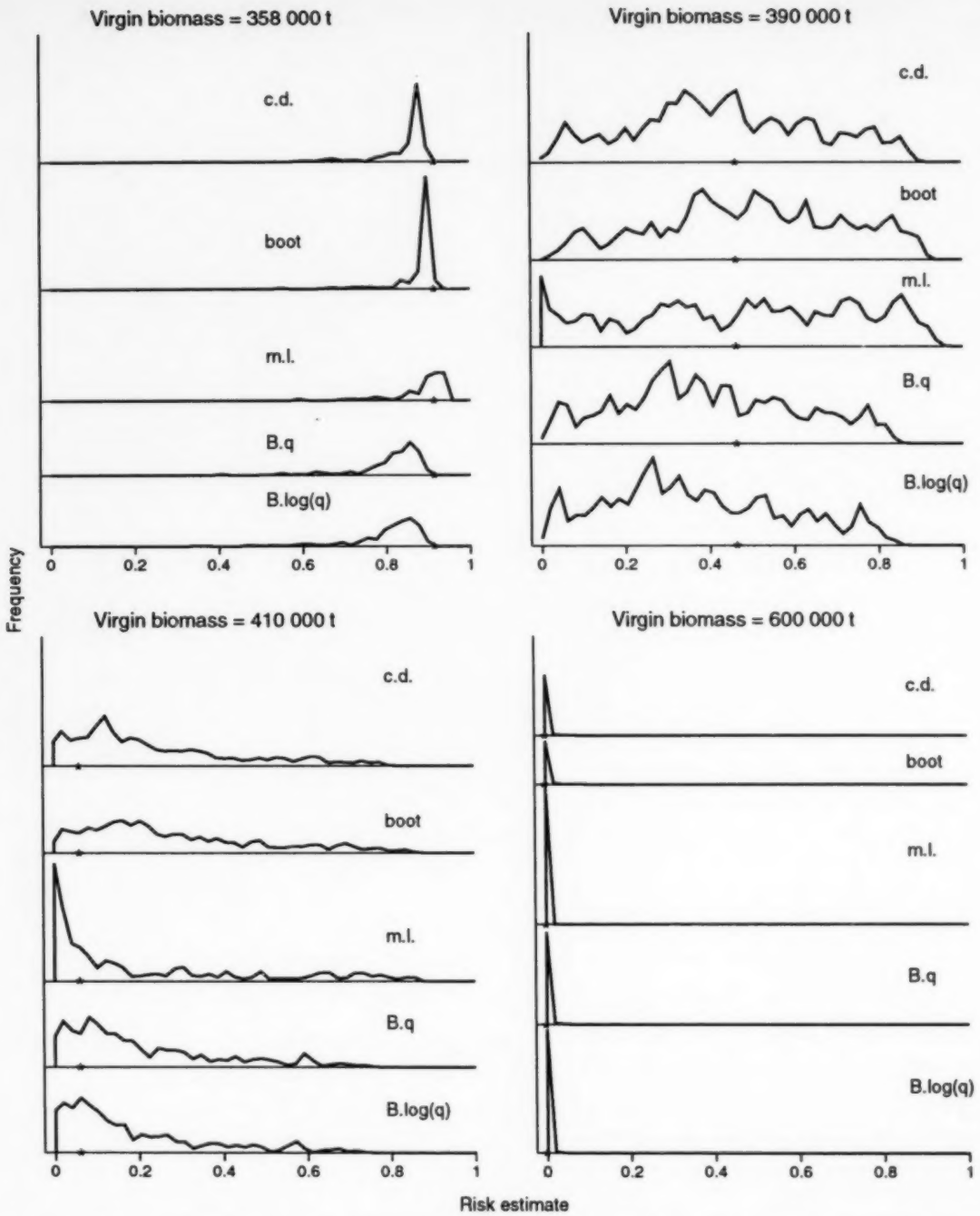

FIG. 3. Simulation results for orange roughy risk function 9. The approximate p.d.f.'s of each risk estimator are shown for given levels of virgin biomass, B_0. The true risk, for each level of virgin biomass, is shown by an asterisk.

estimate low risks and underestimate high risks. Second, the hoki estimators are more badly biased than the orange roughy estimators. Third, the maximum likelihood (m.l.) estimator has the smallest bias overall. Fourth, the other four estimators can be ranked from most optimistic (lowest risk estimates) to least optimistic (highest risk estimates) as follows: Bayesian with uniform distributions for each $\log(q_i)$ (B.log(q)), Bayesian with uniform distributions for each q_i (B.q), confidence distribution (c.d.), and bootstrap. (The relative ordering of the two Bayesian estimators reflects

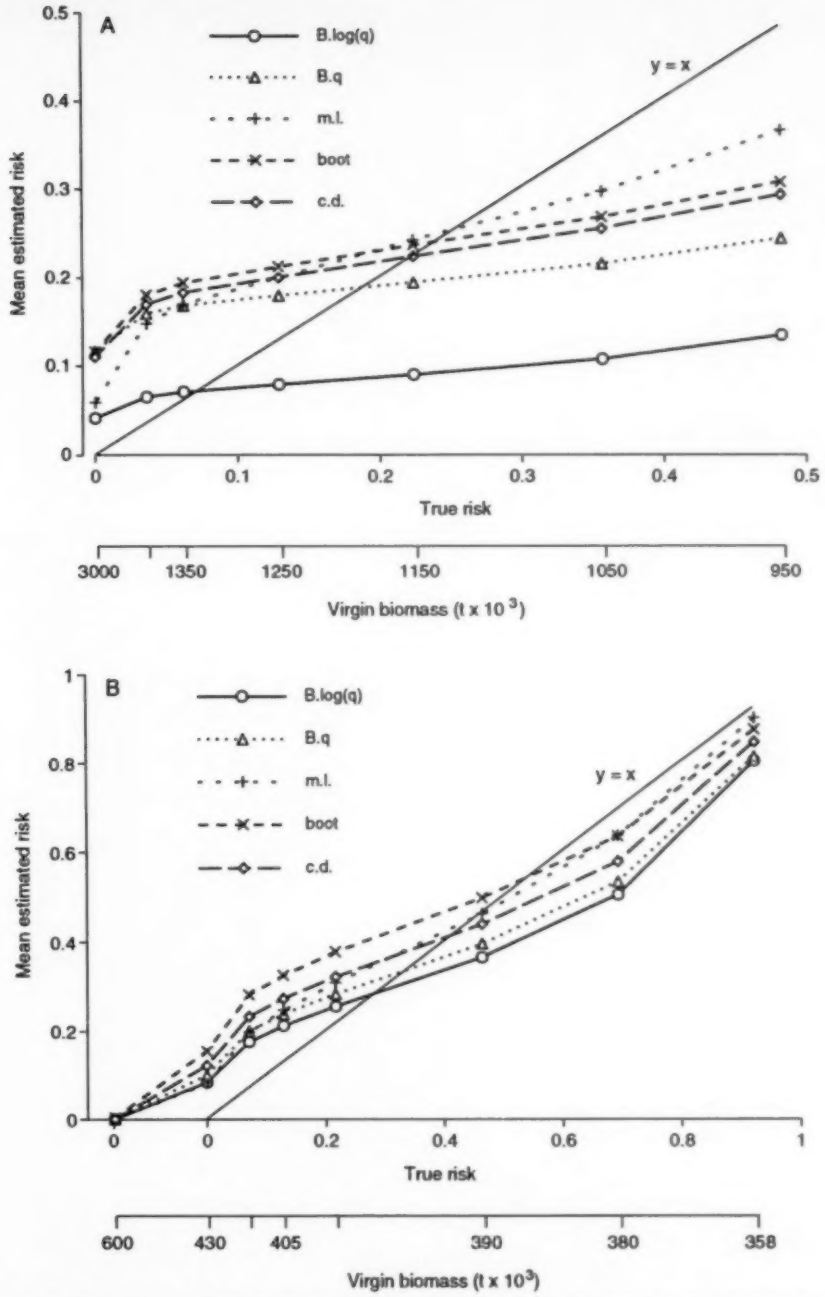

FIG. 4. Mean estimated risk, for each estimator, plotted on a true risk scale, as a function of virgin biomass, for (A) hoki risk function 1 and (B) orange roughy risk function 9.

the relative probabilities accorded to low q_i by their prior distributions. B.log(q) is more optimistic than B.q because the greater weight its prior distribution places on low values of q_i results in greater weight on low values of risk in the posterior risk distribution.)

The ranking of the estimators (excluding m.l.) is reflected in their mean squared error (m.s.e.) (Fig. 5). Thus the most optimistic estimator (B.log(q)) has the lowest m.s.e. when the true risk is low and the highest m.s.e. when it is high. The converse holds for the least optimistic estimator (bootstrap). For hoki, both B.log(q) and m.l. perform markedly worse than the other estimators in terms of m.s.e.; the latter has very high m.s.e. overall. For orange roughy, it is difficult to choose between the estimators in terms of m.s.e. (Fig. 5B).

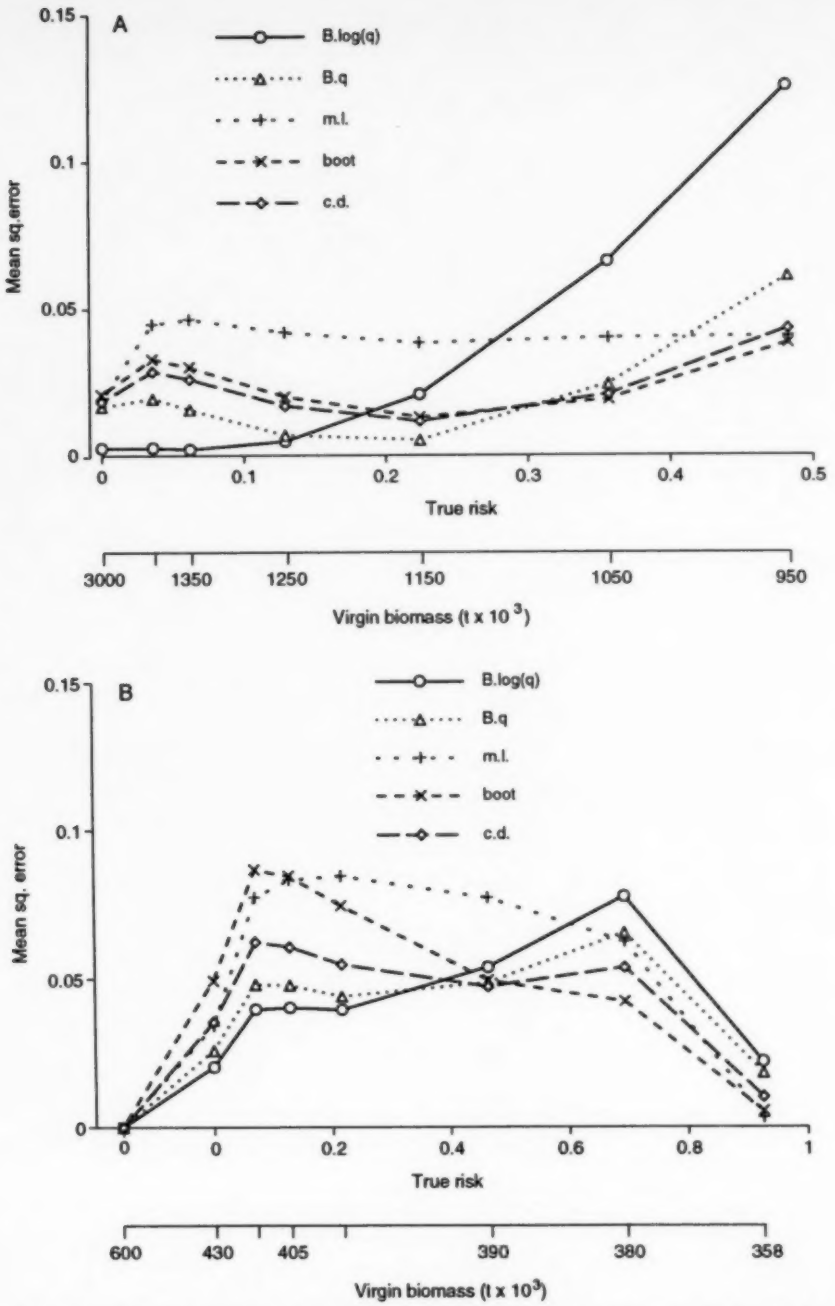

FIG. 5. Mean squared error, for each estimator, plotted on a true risk scale, as a function of virgin biomass, for (A) hoki risk function 1 and (B) orange roughy risk function 9.

There are, however, two points to note. First, m.l. is worse than c.d. and B.*q*, except when the risk is very high. Second, on a minimax basis, c.d. is best with B.*q* a close second.

In terms of distinguishing between high and low risk, none of the estimators perform very well (Fig. 6 shows typical results). Their overall performance can be quantified by considering how each estimator performs when the true risk is low (0.05) or high (0.35). A good estimator would, when the true risk was 0.05, have nearly a 100% chance of returning a low risk and almost no chance of returning a high risk. Similarly, if the true risk was 0.35, a good estimator would almost never give a low risk estimate and would return a high risk estimate nearly 100% of the time.

The nature of the estimators, for this measure of perfor-

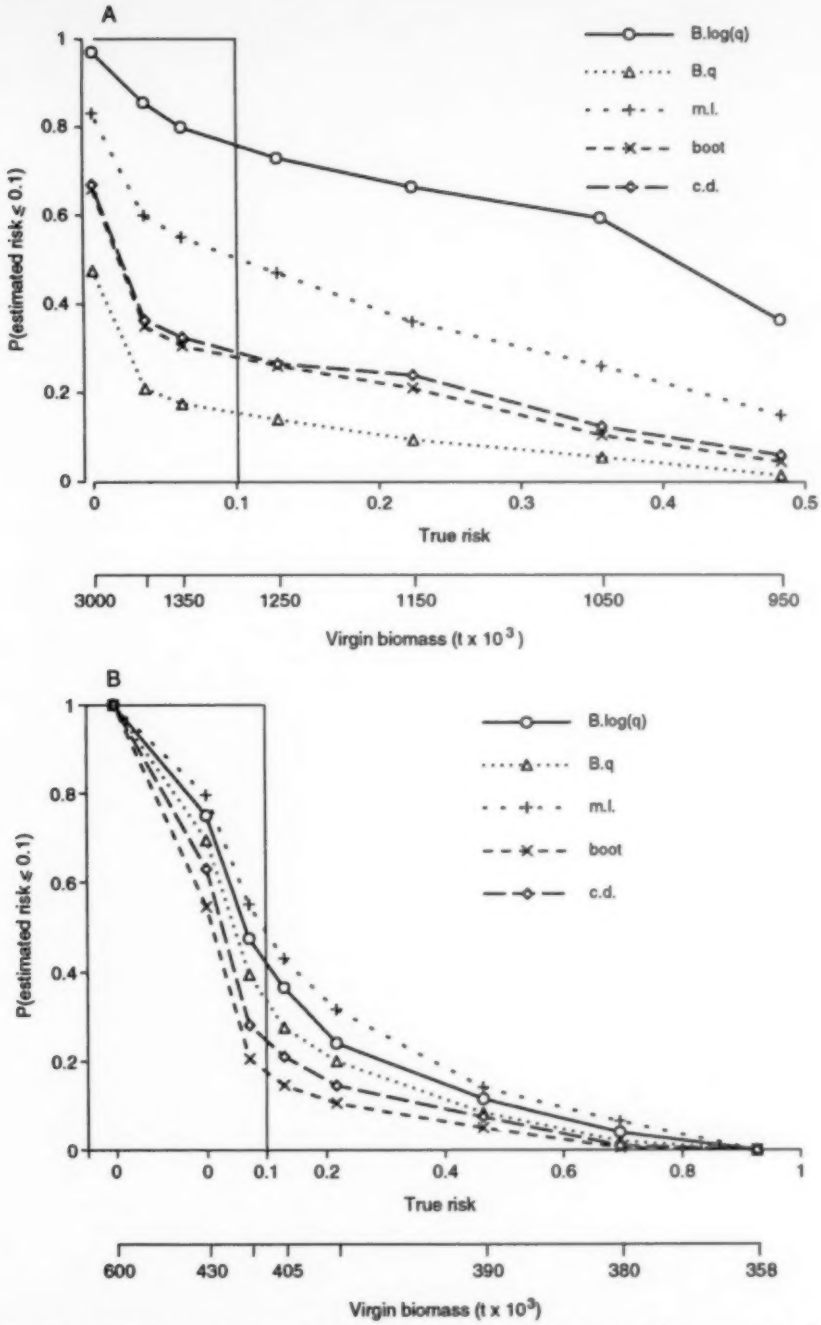

FIG. 6. Probability, for each estimator, that the estimated risk will be low (less than or equal to 0.10) as a function of virgin biomass, plotted on a true risk scale, for (A) hoki risk function 1 and (B) orange roughy risk function 9. The continuous solid lines show the corresponding graph for a perfect estimator.

mance, differs between fisheries (Table 2). For hoki, there are a number of points of note. Blog(q) performs very well when the risk is low, simply because it always returns a low risk estimate (Fig. 2). The B.q estimator has a low probability of returning a low or high risk estimate. This is because it typically returns a "moderate" risk estimate irrespective of the true level of risk (Fig. 2). In contrast, m.l. typically returns either a low risk estimate or a high risk estimate. While it has respectively a 57 and 56% chance of correctly diagnosing a high or low risk, it also has a 27%

TABLE 2. Probabilities of each risk estimator returning a low risk estimate (≤0.10) or a high risk estimate (>0.30) when the true risk is high (0.35) or low (0.05). Corresponding probabilities for a perfect estimator are shown in the bottom row.

	True risk low		True risk high	
Estimator	*P*(low)	*P*(high)	*P*(low)	*P*(high)
Hoki				
B.log(q)	0.83	0.00	0.60	0.00
B.q	0.19	0.01	0.06	0.08
m.l.	0.57	0.27	0.27	0.56
c.d.	0.34	0.19	0.13	0.45
Boot	0.33	0.21	0.11	0.49
Orange roughy				
B.log(q)	0.56	0.15	0.17	0.46
B.q	0.48	0.19	0.14	0.50
m.l.	0.62	0.22	0.22	0.57
c.d.	0.38	0.24	0.11	0.60
Boot	0.31	0.30	0.08	0.69
Perfect	1.00	0.00	0.00	1.00

chance of classifying a high risk as a low risk or a low risk as a high risk. The hoki bootstrap and c.d. estimators behave very similarly and their behaviour falls somewhere between those of the B.q and m.l. estimators.

The results for orange roughy suggest that good performance in one of the four classifications of Table 2 can only be obtained at a loss of performance in another. For example, the bootstrap estimator has a high probability (0.69) of correctly classifying high risk, but has a correspondingly high probability (0.30) of incorrectly classifying a low risk as a high risk. Similarly, it is unlikely (0.08) to return a low risk estimate when the true risk is high, but also has a poor chance (0.31) of returning a low risk estimate when the risk is in fact low. The c.d. estimator behaves like the bootstrap estimator, performing well when the risk is high and performing badly when the risk is low. It is, however, not as extreme as the bootstrap estimator. In contrast, the two Bayesian estimators have lower probabilities of success in classifying high risk, with correspondingly lower probabilities of incorrectly classifying low risk. They also have higher probabilities of correctly classifying low risk at the cost of higher probabilities of misclassifying high risk.

Discussion

Comparison between Fisheries

In the hoki case, our information is poor: the time series of biomass indices are short and their c.v.'s are large (Table 1). Thus, we would expect our risk estimators to be relatively poor. Indeed, two of the estimators are very poor.

The B.log(q) estimator invariably returned a low risk estimate, irrespective of the true risk. Its behaviour as an estimator was unacceptable in terms of bias, m.s.e., and its ability to distinguish between low and high risk. The m.l. estimator performed adequately except in terms of m.s.e. It had a relatively constant m.s.e. which was almost everywhere higher than that of the other estimators (except B.log(q)).

The three remaining estimators, while better than the alternatives, are still not good in absolute terms. The bootstrap and c.d. estimators perform reasonably when the risk is high, but when the risk is low, they most often return a moderate risk estimate. The B.q estimator performed adequately on bias and m.s.e. but had virtually no ability to distinguish between high and low risk. It usually returned a moderate risk estimate.

We might expect our estimators to perform better for orange roughy than for hoki because we have a longer time series of biomass indices with much lower c.v.'s (Table 1). This turns out to be true in some respects but not in others.

Certainly the two Bayesian estimators have much more desirable properties in the orange roughy case. When compared with the other three estimators, they have different but comparable properties in terms of bias and m.s.e. and there are no reasonable grounds on which they can be rejected as estimators.

In terms of bias the orange roughy estimators are all improved (Fig. 4), but in terms of m.s.e. the estimators perform less well (Fig. 5). The typical m.s.e. for each estimator (except B.log(q)) is much larger for orange roughy than for hoki. This is consistent with our earlier suggestion that the difference between the risk functions for the two fisheries (Fig. 1) should make it intrinsically more difficult to estimate risk for orange roughy than for hoki.

One way to account for the different properties of the risk functions, while comparing the levels of m.s.e., is to look at the levels relative to those which could be achieved without any information about B_0. Without any sample data the best hoki risk estimate, on a minimax basis, would be $0.48/2 = 0.24$ which has a maximum m.s.e. of $(0.24)^2 = 0.06$. Similarly the best orange roughy estimate would be $0.93/2 = 0.465$ which has a maximum m.s.e. of $(0.465)^2 = 0.22$. Comparing the maximum m.s.e.'s of, for example, the c.d. estimator, we have 0.045 for hoki and 0.06 for orange roughy. It is clear that in the orange roughy case the c.d. estimator has moved further from the best "no information" estimator than it has in the hoki case.

In terms of ability to differentiate between high and low risk the Bayesian estimators are much better for orange roughy than for hoki (Table 2; Fig. 6). However, in an absolute sense, they are still not very good, classifying low risk as high or high risk as low about 15% of the time. The m.l. estimator performs fractionally better in the orange roughy case, but in an absolute sense is certainly no better than the orange roughy Bayesian estimators.

The bootstrap and c.d. estimators in one sense perform better in the orange roughy case. That is, they perform better when the true risk is high. However, they have a quite unbalanced behaviour when the true risk is low, being almost as likely to return a high risk estimate, in that situation, as a low risk estimate (Table 2). This is particularly true of the bootstrap estimator.

Implications for the 1991 Stock Assessments

It is clear from the results that all of the estimators considered in this paper are far from precise. At the time they were applied in the 1991 New Zealand stock assessments the precision of the estimators was not known. The results in this paper do not change the conclusion of either assessment, but our confidence in the conclusions is reduced.

The conclusion of the hoki stock assessment (Cordue et al. 1992) was that there was little short-term risk to the

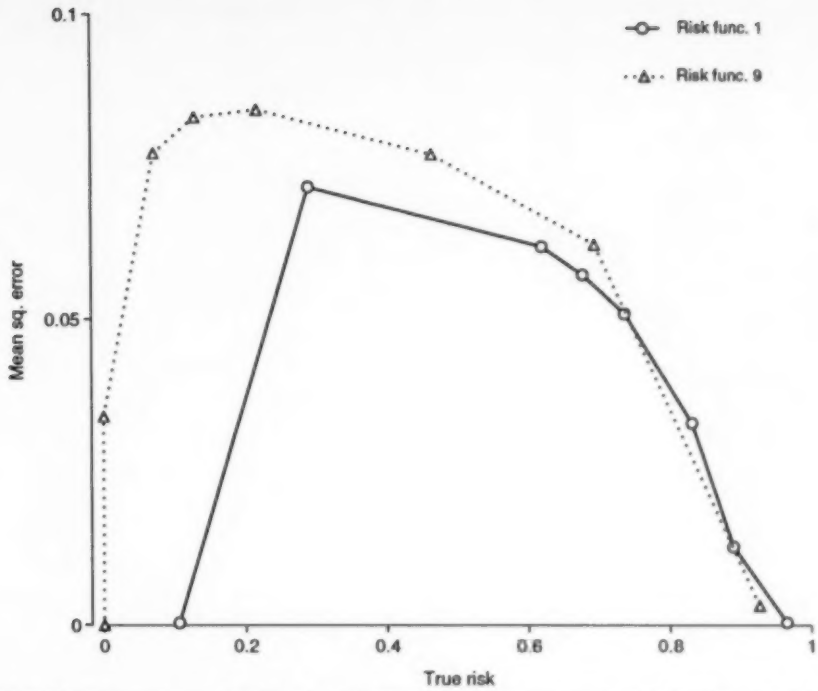

FIG. 7. Mean squared error for the m.l. estimator of risk for orange roughy risk functions 1 and 9.

fishery and that any necessary action could be delayed until more data had been collected. Now, we cannot be sure that the risks involved are not much higher than were estimated. The actual c.d. risk estimate was 0.29: but if the true risk is 0.4, we would expect a risk estimate of 0.29 or less 45% of the time, and if the true risk is 0.48 (the maximum possible), we would expect a risk estimate of 0.29 or less 33% of the time.

The Chatham Rise orange roughy assessment (Francis and Robertson 1991) concluded that the current policy of a 5000-t annual decrease in quota was too risky. The c.d. estimate of risk was 0.54: although not precise, the estimate is so large that it is unlikely that the true risk is in fact low. If the true risk were zero, we would only observe a risk estimate of 0.54 or greater about 2% of the time; if the true risk were 0.25, we would still only get such a high risk estimate 18% of the time.

Choice and Accuracy

In terms of absolute m.s.e. the orange roughy risk estimators performed less well than the corresponding hoki risk estimators. This was due to the different natures of the risks they were trying to estimate. An examination of the corresponding risk functions showed that the hoki risk functions were substantially less steep than the orange roughy risk functions, and therefore, risks in the hoki fishery were easier to estimate.

This observation can be generalised and in some circumstances used to advantage. If, for a given fishery, there is some flexibility in the types of risk which can be defined, then analysts can choose definitions which result in a set of relatively shallow risk functions and so improve the accuracy of their estimates.

As an example, consider the four risk functions presented in this paper for the orange roughy fishery (Figs. 1B, and 1C). The first figure presents risk functions for two management strategies, for one type of risk, while the second figure shows the corresponding functions for another type of risk. It is clear that for the first type of risk the functions are shallower. We would therefore expect that the risk estimators for the first type would be more precise. Indeed, this is the case (Fig. 7). There is little difference in the m.s.e. for high values of risk, but there is an increasing difference as the risk approaches zero. This trend reflects the relative steepness of the risk functions for different values of true risk.

Of course, the failure of any of the assumptions of the age-structured model underlying the risk estimation could also decrease accuracy.

Conclusions

The results in this paper draw attention to two problems that analysts must consider when attempting the type of risk analysis described earlier: the choice of an analytical technique (which *D*-estimator? which type of risk?) and the likely accuracy of the risk estimator. Although a number of authors have estimated risk in a fisheries setting (see references in the introduction), we are unaware of any previous attempt to consider the accuracy of risk estimates.

Our results provide some help in the problem of choosing a *D*-estimator. First, the use of the m.l. estimator seems unwise. Second, the fact that our four *D*-estimators show the same ranking (from most optimistic to least optimistic) for both fisheries, and all risk functions considered, gives information about the relative consequences of choosing one estimator rather than another. In particular, the ques-

tion of which is the best estimator depends on whether it is more serious to estimate high risk when the true risk is low or to estimate low risk when the true risk is high. However, this in turn depends on how risk estimates are used to make management decisions and how success is measured for a given fishery. In other words, to fully resolve the question of which estimator to use, we need the full apparatus of decision theory (Ferguson 1967; Berger 1985). (However, it seems unlikely that the very optimistic behaviour of the B.log(q) estimator for the hoki fishery would ever be useful.)

Our reformulation of risk analysis, specifically the definition of a risk function, may be of help where there is some choice in how to define risk. The steeper the risk function, the less accurate the estimate of risk will be.

The inaccuracy of all of the risk estimators in the examples considered in this paper provides a warning to analysts. While it is useful to phrase advice to managers in terms of the risk to the fishery of various options, it also seems necessary to advise them as to how well those risks have been estimated. How best to do this is a question which needs addressing, but however the advice is conveyed, it seems clear that a manager should treat "poor" (i.e., imprecise and biased) risk estimates differently from "good" risk estimates. This is an issue that has not been addressed (as far as we are aware) in the decision theory literature. In discussing the meaning of "risk", we mentioned two elements that are often considered: the probability that something bad will occur and the magnitude of the consequences. Perhaps we should adopt Mendelssohn's (1979) suggestion that uncertainty about the probability is a further source of risk.

Acknowledgments

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Appendix: Alternative Ways of Calculating $D(I)$

Here we describe the four ways of calculating $D(I)$ that are used in this paper: confidence distribution, Bayesian, maximum likelihood, and bootstrap.

The confidence distribution method used here differs from that of Francis (1992) in only one respect: we assume that the c.v.'s of the biomass indices are known. This alters only the likelihood function; the application of the method remains the same.

The Bayesian approach used by Cordue et al. (1992) involves specifying a joint prior distribution for the unknown parameters (B_0 and the q_i). This distribution is supposed to describe the analyst's belief about the value of the parameters before the data (the biomass indices) were observed. From this prior distribution, a posterior distribution is calculated in the standard Bayesian manner. Finally, $D(I)$ is calculated as the marginal posterior on B_0 by integrating the posterior with respect to the q_i. This integration would normally be done numerically. However, for this particular problem, an approximation allows direct calculation of the integral (details available from the authors).

In the maximum likelihood approach, also used by Cordue et al. (1992), $D(I)$ is simply the special distribution which has

a point mass of 1 at $\hat{B}_0$ where $\hat{B}_0$ is the maximum likelihood estimate of B_0 described by Francis (1990, 1992). Thus, the associated D-estimator is simply $r_s(\hat{B}_0)$. Our naming of this estimator as "maximum likelihood" needs clarification. In general, if X is the maximum likelihood estimator of a real parameter θ, and g is a one-to-one real function, then $g(X)$ is the maximum likelihood estimator of $g(\theta)$. When g is not one-to-one, $g(X)$ may or may not be a maximum likelihood estimator, but is typically used as such in any case (Scholz 1985). Because the function r_s may not be one-to-one, our estimator $r_s(\hat{B}_0)$ is a maximum likelihood estimator only in this loose sense.

A fourth way of calculating $D(I)$ is via the bootstrap (Efron and Gong 1983). The bootstrapping method used in this paper is as follows: (i) calculate $\hat{B}_0$ from the set of observed biomass indices I, (ii) use $\hat{B}_0$ as the starting biomass in the stock reduction model and run the model to calculate the biomass for each year that an index occurs in I, (iii) simulate a set of biomass indices J (with the same years represented and containing the same number of indices as I) using the biomasses calculated in step (ii) and the known c.v.'s of the indices in I, (iv) treating J as a set of observed biomass indices, calculate and store $\hat{B}_0^*$ (this is a simulated estimate), and (v) repeat steps (iii) and (iv) a large (e.g., 200) number of times. The bootstrap distribution, $D(I)$, is the empirical distribution of the $\hat{B}_0^*$ values stored at step (iv).

Muscle Tissue Growth and Muscle Fibre Dynamics in the Tropical Loliginid Squid *Photololigo* sp. (Cephalopoda: Loliginidae)

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Moltschaniwskyj, N.A. 1994. Muscle tissue growth and muscle fibre dynamics in the tropical loliginid squid *Photololigo* sp. (Cephalopoda: Loliginidae). Can. J. Fish. Aquat. Sci. 51: 830–835.

Growth of somatic tissue in *Photololigo* sp. was examined in terms of muscle fibre recruitment and growth. Muscle blocks and muscle fibres were measured and size frequency distributions compared between different size-classes of squid. Muscle blocks increased in size as individuals grew. The size frequency distribution of the muscle blocks suggested that this increase was due to both the generation of new muscle fibres and an increase in the size of existing muscle fibres. The size frequency distribution of muscle fibres was very similar in all size-classes of squid examined, and the presence of small muscle fibres in all individuals suggested that fibre recruitment may be continuous. Growth of muscle tissue, by muscle fibre growth and recruitment, provides a mechanism to explain constant growth throughout the life cycle described for tropical squid. Two structural types of muscle fibres, mitochondria-poor and mitochondria-rich, are present in juvenile and adult squid. A weak relationship between the ratio of the two muscle fibre types and dorsal mantle length suggests that generation of mitochondria-rich fibres may not be influenced by growth.

Le développement des tissus somatiques chez *Photololigo* sp. a été étudié en termes de grossissement et de multiplication des fibres musculaires. Des faisceaux de muscles et des fibres musculaires ont été mesurés et les distributions par fréquences de taille de calmars selon différentes classes ont été comparées entre elles. Les faisceaux musculaires augmentent de volume à mesure du développement des sujets. La distribution par fréquence de taille des faisceaux musculaires semble indiquer que l'augmentation observée est attribuable à la fois à la production de nouvelles fibres musculaires ainsi qu'au grossissement des fibres musculaires existantes. Celle des fibres musculaires est très homogène dans toutes les classes de taille de calmar examinées, et la présence de petites fibres musculaires chez tous les sujets indiquerait que l'apparition de nouvelles fibres est un phénomène continuel. Le développement du tissu musculaire, par production et par grossissement des fibres musculaires, est un mécanisme qui permet d'expliquer la croissance constante de ce calmar tropical jusqu'au bout de son cycle biologique. On observe deux types structuraux de fibres musculaires, soit celles qui sont riches et celles qui sont pauvres en mitochondries; elles sont observées chez le calmar juvénile comme chez le calmar adulte. L'existence d'une relation peu marquée entre la proportion des deux types de fibres musculaires l'un par rapport à l'autre, et la longueur dorsale du manteau, paraît indiquer que la production des fibres musculaires riches en mitochondries peut ne pas être influencée par la croissance.

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The live-fast die-young lifestyle of cephalopods has attracted considerable attention, with the result that a suite of growth curves has been produced (Forsythe and Van Heukelem 1987). The literature suggests that squid growth may consist of two growth stanzas: a period of exponential growth followed by a logarithmic growth phase (Forsythe and Van Heukelem 1987). However, there is also evidence that numerical models used to generate values for growth patterns may have methodological problems (Alford and Jackson 1993). Instead, growth that continues throughout the life cycle may be a more typical scenario for squid. Both linear and exponential growth relationships have recently been described for squid (e.g., ommastrephid squid, Rodhouse and Hatfield 1990; Arkhipkin and Mikheev 1992; tropical loliginid squid, Jackson and Choat 1992). Overall growth rates of squid exceed those of fish because they do not appear to cease growing. The capability of squid to achieve such a high growth rate has been met with some scepticism, with the suggestion that cephalopods to not have the physiological requirements to grow rapidly (Jarre et al. 1991). However, the underlying assumption is that the mechanics of somatic growth are comparable between squid and fish.

The expression of growth as an increase in somatic tissue, usually weight or length, is common throughout the literature (e.g., Ricker 1979; Forsythe and Van Heukelem 1987; Weatherley and Gill 1987). Increases in mass and length are a function of muscle tissue growth, a process that occurs at the cellular level. For both fish and squid, muscle tissue comprises approximately 90% of the body mass. Therefore, an understanding of muscle fibre dynamics should enable us to understand how growth occurs. Muscle growth in teleosts occurs via two mechanisms: (1) initial recruitment of new muscle fibres (hyperplasia) that causes rapid growth and (2) an increase in muscle fibre size (hypertrophy) as the capability of generating new muscle fibres diminishes, resulting in slower growth (Weatherley and Gill 1985). The physiological constraint of surface area to volume ratios restricts the maximum size of a muscle fibre. The final, or asymptotic, size fish may achieve will be determined by the number of muscle fibres present once hyperplasia ceases

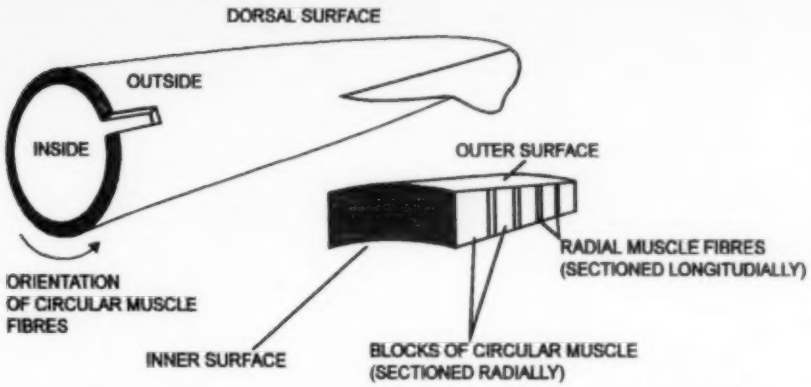

FIG. 1. Schematic diagram of a squid showing where the muscle block (rotated 90°) was removed and the orientation of the muscle fibres examined.

(Weatherley and Gill 1985). Individuals with more muscle fibres have a greater potential to reach a larger size.

For squid, there is little detail on how the dynamics of muscle fibre recruitment and growth change during somatic growth. Descriptions of squid mantle muscle have largely contributed to an understanding of locomotor function and operation (Ward and Wainwright 1972; Packard and Trueman 1974; Moon and Hurlbert 1975; Bone et al. 1981; Gosline et al. 1983). Measures of muscle fibre size and density have received attention in only a few cases (*Alloteuthis*, *Loligo*, and *Sepia*, Bone et al. 1981; *Illex argentinus*, Hatfield et al. 1992). Variation in muscle fibre arrangement and size throughout the mantle muscle has not been examined.

Photololigo sp. is the most abundant loliginid squid commonly found in the Townsville region, on the north-east coast of tropical Australia. This species has been referred to as *Loligo chinensis*; however, recent electrophoretic analysis has identified this species as currently undescribed, belonging to the genus *Photololigo* (Yeatman and Benzie 1994). This paper examines how the dynamics of muscle fibres determine growth in juvenile and adult *Photololigo* sp. Given that growth in *Photololigo* sp. does not slow down (Jackson and Choat 1992), exponential growth may be due to continuous muscle fibre generation. An examination of both juveniles and adults will provide a full spectrum of sizes, thereby allowing an assessment of how growth relates to changes in muscle fibre size, number, and structural state. By examining the muscle fibre dynamics in squid species, an insight into how growth is occurring and the limiting factors on growth rate and ultimate size is possible.

Materials and Methods

Muscle Tissue Collection and Preparation

Juvenile *Photololigo* sp. (2.5–40 mm dorsal mantle length) were collected, using automated light-traps (Doherty 1987) in the Cleveland Bay region between October and January of the 1991–92 austral summer. Adult *Photololigo* sp. (40–150 mm dorsal mantle length) were caught in the same area using paired otter trawls (4 cm mesh) between August and November 1991 and in March 1992.

Dorsal mantle length (DML) of each individual was measured before fixation of tissue. Juveniles were fixed whole and a sample of muscle tissue was removed later. From each adult, a sample of muscle tissue was removed for fixation. All muscle tissue was fixed in a formalin – acetic acid – calcium chloride solution (10 mL of 37% formaldehyde, 5 mL of glacial acetic acid, 1.3 g of calcium chloride (dihydrate), and distilled water to 100 mL). Fixed material was transferred to 70% ethanol 48 h before processing. Muscle tissue was dehydrated through an ascending isopropanol series, cleared in chloroform, and infiltrated in paraffin wax (Paramat). Tissue blocks were sectioned at 7 μm, decerated in xylene, and hydrated through a descending ethanol series. Histological sections were stained with the trichrome Mallory-Heidenhain stain.

Squid mantle tissue is predominantly made up of circular smooth muscle fibres separated into blocks by thin regions of radial muscle tissue (Ward and Wainwright 1972). These smooth muscle fibres have helical myofibrils and are typically small, <10 μm in diameter (Hanson and Lowy 1957). In many cephalopods, circular muscle fibres are present in two structural states, mitochondria-rich and mitochondria-poor, analogous to fast and slow twitch muscle fibres in vertebrates (Mommsen et al. 1981). Muscle tissue was sectioned longitudinally so that circular muscle fibres, i.e., those muscle fibres that encircle the muscle mass, were sectioned radially and radial muscle fibres were cut longitudinally (Fig. 1). The bulk of the mantle muscle tissue is made up of circular, mitochondria-poor muscle fibres. Therefore, these muscle fibres were used in assessing muscle fibre dynamics and mantle muscle growth. The dynamics of muscle fibre recruitment and growth were described by examining size frequency distributions of circular muscle blocks and circular muscle fibres.

Sections of muscle tissue were examined and measures made using computer-aided image analysis at 160 and 400× (oil immersion). An Olympus CH-2 high-power microscope connected to an Apple Macintosh IIci computer displayed images using a Rasterops 364 video colour card. Images were stored using "Framegrabber" software and analysed using the "NIH IMAGE" program (public domain software).

Pilot Study

Since little is known about the dynamics of muscle growth in squid, it was necessary to determine if and how muscle fibre dynamics and growth differed between areas of the mantle muscle tissue. This would allow me to standardise the

TABLE 1. ANOVA table for the pilot study examining variation in circular muscle block widths between individuals, around the mantle muscle, and along the longitudinal axis.

Source	df	Sum of squares	Mean squares	*F*	Probability
Individual	4	0.3509	0.0877	1461.67	0.0000
Distance around the body	4	0.0007	0.0002	3.33	0.0101
Area along the body	2	0.0616	0.0308	513.33	0.0000
Individual × distance	16	0.0066	0.0004	6.67	0.0000
Individual × area	8	0.0454	0.0057	95.00	0.0000
Distance × area	8	0.0047	0.0006	10.00	0.0000
Individual × distance × area	32	0.0244	0.0008	13.33	0.0000
Error	1347	0.5829	0.00006		

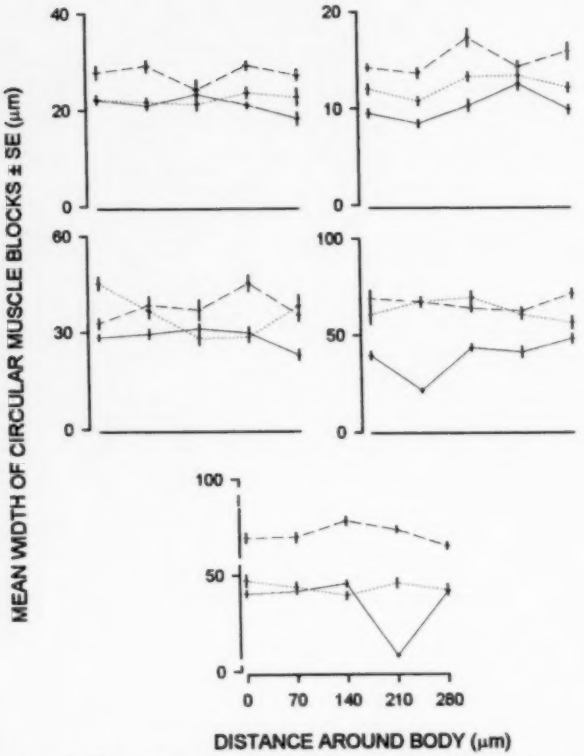

FIG. 2. Circular muscle block widths for five individuals in the pilot study, showing how the average muscle block width changes with distance around the body and the area along the body. Solid line = posterior, dotted line = middle, and dashed line = anterior. Each point is the mean of 20 circular muscle blocks. The five graphs represent each individual used in the pilot study.

region of the mantle from which to sample muscle tissue from each individual, thus allowing comparisons between individuals. Five individuals (5.9–16.7 mm DML) were sectioned longitudinally through the entire body cavity. A section was selected and designated distance zero. Points 70, 140, 210, and 280 μm around the body from this starting point were selected for measurement (these will be referred to as "distances" around the body). The longitudinal axis of individuals was divided into three "areas": anterior, middle, and posterior. From each area and distance in every individual, 20 measurements of circular muscle block width were taken. In this way, it was possible to determine the variation in block width among individuals, among distances around the body, and among areas. These measurements allowed me to decide where measurements of block widths should be taken for comparison of different sized individuals.

Muscle Block and Muscle Fibre Size Measurements

Results from the pilot study indicated considerable variability in the size of muscle fibres and muscle blocks within an individual. There was no apparent trend in change in muscle block or muscle fibre size, either around the body or along the longitudinal axis (see Results). It was therefore decided to standardise the location from where tissue was removed. The cartilaginous mantle locking mechanism was used as a standard location on the mantle muscle. Tissue samples were removed from the dorsal side of the mantle, at a point level with the locking mechanism. Given the variability within an individual, it was calculated that measurements of 42 circular muscle blocks would provide the sample size necessary to detect differences between individuals. From the muscle tissue sample from each individual, seven histological sections were prepared and seven randomly chosen circular muscle blocks from each histological section were measured.

To obtain adequate numbers of muscle fibre diameters for size frequency analysis, 60 muscle fibres were measured from each individual. As the muscle fibres are fusiform in shape and oval shaped in cross-section, the longest diameter of each muscle fibre was measured. Muscle fibres were randomly selected for measurement from three different circular muscle blocks for each squid.

Type of Muscle Fibres

Circular muscle tissue is present in mitochondria-rich and mitochondria-poor states. Changes in the ratios of the two muscle fibre types were examined during growth. Mitochondria-rich fibres are present at two distinct locations in the mantle muscle tissue, along the inner and the outer surfaces of the mantle tissue. Ten measurements of the width of the mitochondria-rich muscle fibre region were taken on each of the inner and outer surfaces for each individual. In addition, 10 measurements of the total width of the mantle muscle were obtained. The width of the mitochondria-poor muscle fibre region could then be calculated. Values were averaged for each individual. Changes in the relative proportion of the two muscle fibre types were examined as a function of DML.

Analyses

Differences in muscle block width in the three factors examined, along the body, around the body, and among individuals, were analysed using a three-factor orthogonal analysis of variance (ANOVA).

The size frequency distributions of the muscle blocks and fibres among the individuals in eight DML groups were compared. Circular muscle block and fibre diameter size frequencies were compared across dorsal mantle length size-classes using a chi-square frequency analysis.

Results

Pilot Study

A significant interaction term in the ANOVA among the three factors (distance along body, distance around the body, and individual squid) indicated differences in block widths within individuals (Table 1). Muscle block widths did not change systematically with either distance around the body or areas along the longitudinal axis (Fig. 2). To obtain comparative information for squid of different DML, muscle tissue was sampled from the same location on all individuals.

Muscle Fibre Blocks

Muscle block widths varied from 7.5 μm in the smallest juveniles to 1187 μm in larger adults. A significant difference was evident in the size distribution of muscle blocks ($\chi^2 = 2561.87$, df = 70, $P < 0.05$) with block widths increasing with growth in DML (Fig. 3). In smaller individuals, there was a predominance of narrow muscle blocks, with new muscle blocks appearing in individuals less than 80 mm DML. Individuals 60–80 mm DML had a mixture of all block widths, although as growth continues, circular muscle blocks increase in width. There was no evidence that new muscle blocks were being added in the region of the mantle muscle tissue examined.

Muscle Fibre Diameters

Muscle fibre diameters ranged from 0.49 to 6.17 μm (Fig. 4). The frequency distribution of muscle fibre sizes differed among the size-classes of squid ($\chi^2 = 370.96$, df = 84, $P < 0.05$). There was a predominance of very small muscle fibre sizes in the smaller individuals (<80 mm DML) (Fig. 4). Larger individuals (≥80 mm DML) had a prevalence of medium-sized muscle fibres. The presence of muscle fibres of <1 μm in all individuals suggests that new muscle fibres were still being generated in larger squid, but there were relatively fewer than in smaller squid.

Mitochondria-Rich Muscle Fibres

In all individuals the mitochondria-rich muscle fibre region was relatively small, comprising less than 1% of the overall mantle tissue width. The ratio of the two muscle fibre types changed as a function of growth in these squid (Fig. 5). The amount of mitochondria-rich tissue decreased relative to the mitochondria-poor tissue with increasing DML. Considerable variability in the width of the mitochondria-rich muscle fibre region between individuals of similar size suggests that the amount of mitochondria-rich muscle fibres may not be a function of increasing mantle length.

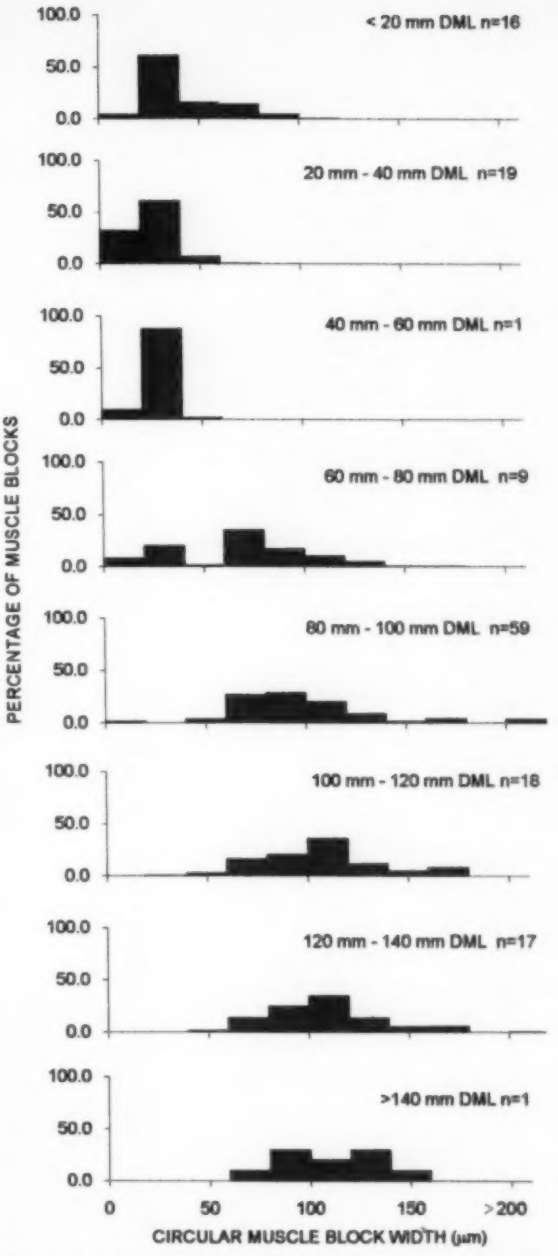

FIG. 3. Size frequency distribution of circular muscle blocks in individuals in different DML size-classes. Forty-two circular muscle blocks were measured from each squid. Midpoints of muscle block size-classes are shown on the *x*-axis; *n* = number of individuals.

Discussion

This examination of muscle fibre dynamics in squid suggests that growth occurs by a combination of hyperplasia and hypertrophy. Growth via these two mechanisms is typical of teleost species (Weatherley and Gill 1985). This

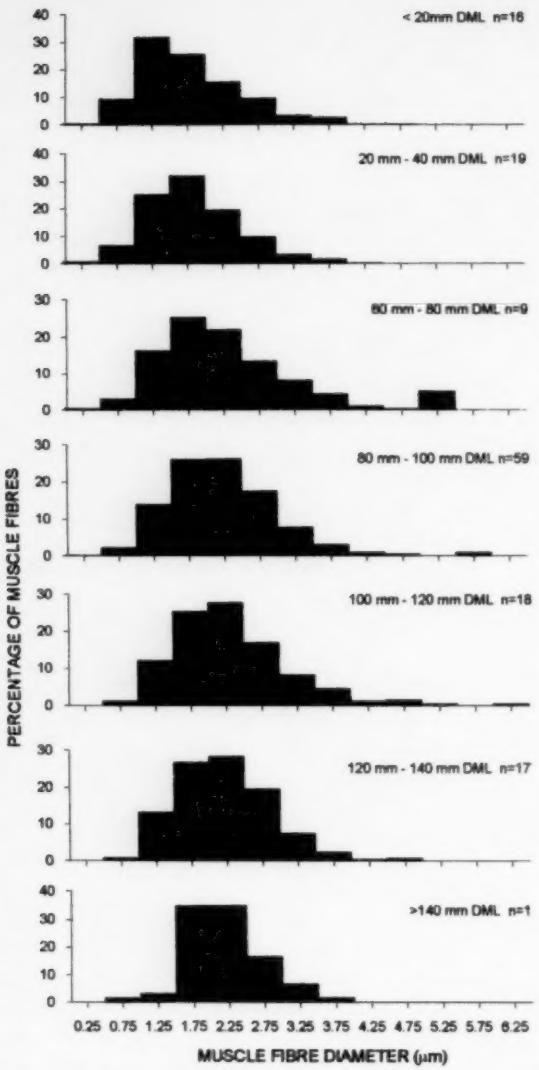

FIG. 4. Size frequency distribution of circular muscle fibres in different DML size-classes of individuals. No squid in the 40–60 mm size-class were sampled. Sixty muscle fibres were measured from each individual. Midpoints of muscle fibre size-classes are shown on the *x*-axis; *n* = number of individuals.

study highlights a major difference between squid and fish: squid appear to have the capacity for continued hyperplasic growth throughout their entire life. Such hyperplasic growth will have the net result of continuous growth, with no maximum size imposed by the physiological constraints of cell size. Accordingly, squid growth cannot be described using mathematical growth models generated for teleost species.

The striated muscle fibres of the mantle tissue are elongate and fusiform, such that the presence of small-diameter muscle fibres may have been due to examining the narrower portion of the muscle fibre. It is not possible to differentiate between small muscle fibres and the ends of muscle fibres. However, two pieces of evidence support the idea that some of the small muscle fibres were recruited. Firstly, as muscle

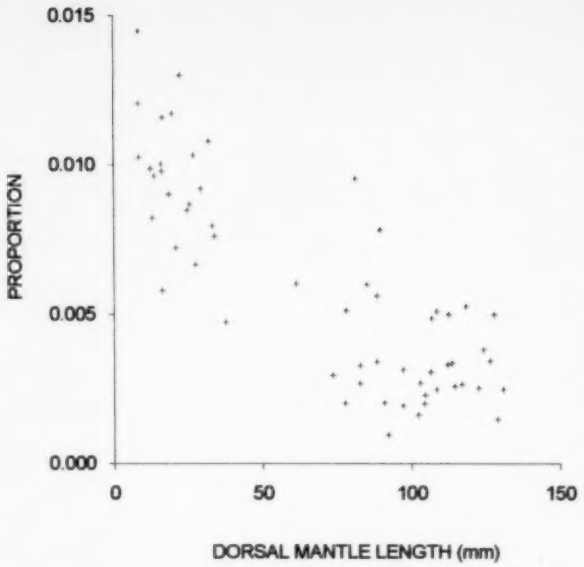

FIG. 5. Ratio of the width of mitochondria-rich and mitochondria-poor muscle fibre areas as a function of DML.

fibres grow they increase both in diameter and length, so the probability of cutting the terminal portion of a muscle fibre becomes reduced, with the result that fewer small diameters, due to fibre ends, will be recorded. Secondly, the oblique striated circular muscle reaches a maximum diameter of 10 μm (Hanson and Lowy 1957; Bone et al. 1981); in this study it was 6.17 μm. Therefore, if muscle fibre recruitment ceased and the existing muscle fibres reached maximum size, the muscle blocks, and therefore the individual, would not continue to increase in size. Yet the circular muscle blocks continued to increase in size as the squid became larger. The evidence collected in this study suggests that muscle fibre recruitment must still be occurring within existing muscle blocks. Hence, growth is accomplished by an increase in muscle fibre number and muscle fibre size in existing muscle blocks throughout the life of *Photololigo* sp.

The absence of small muscle blocks in large squid suggests that new circular muscle blocks do not arise within the region of the tissue examined. Circular muscle blocks at the anterior end of the animal were better defined when compared with muscle blocks in the posterior region. Muscle blocks may be created at the anterior end, presumably by the recruitment of both circular and radial muscle fibres. There is a need to further examine whether new muscle blocks are being created in larger squid and how much their production contributes to growth.

The phenomena of sustained muscle fibre recruitment and minimal increase in muscle fibre size have been observed in juveniles of large, fast-growing teleost species (Weatherley et al. 1988). Weatherley et al. (1988) attributed the maximal size of fish species to the relative periods of hyperplasic and hypertrophic growth of muscle fibres. It may be possible to examine differences in squid growth in the same light. *Photololigo* sp. is a relatively small squid compared with its large muscular oceanic relatives. It would be interesting to ascertain the relative contribution of hyperplasic

and hypertrophic growth in such species. It is possible that larger squid species are using hyperplasic growth to a greater extent to attain larger sizes. Unfortunately, tissue sampling and histological techniques can influence muscle fibre size. Given the lack of standard techniques for tissue collection and preservation, comparisons of muscle fibre dynamics in squid mantle muscle tissues between studies are currently limited.

With little in the way of lipid and glycogen reserves, the muscle tissue of squid also serves as an energy reservoir (O'Dor and Webber 1986). Hence, changes in muscle tissue may provide a tool to assess the effect of nutrition on growth. Weatherley et al. (1980) examined juvenile fish reared under differing temperature and feeding regimes. They recorded little difference in the size frequency structure of the muscle fibres, until hyperplasia ceased. From these results, Weatherley et al. (1980) postulated that in juveniles the relative rates of hyperplasia and hypertrophy are constant. In other words, it is not possible to assess how conditions are affecting somatic growth by examining muscle fibre size distributions during a period of muscle fibre recruitment. If in squid the relative rates of hyperplasia and hypertrophy are constant throughout the life cycle, then the only way to detect the effects of differing growth regimes will be by muscle block measurements. Clearly there is a need to expose cultured squid to a variety of rearing regimes and examine the effects on muscle fibre dynamics, muscle block dynamics, and growth.

The structural makeup of the muscle tissue changed little during growth. Both mitochondria-rich and mitochondria-poor muscle fibre types were present throughout the life of *Photololigo* sp. The decreasing proportion of mitochondria-rich tissue with increasing DML indicates that increasing muscle bulk is predominantly a function of mitochondria-poor muscle tissue. Mitochondria-rich muscle fibres are restricted to the inner and outer surfaces of the mantle muscle and are predominantly involved in slow, steady-state swimming and respiratory movements (Mommsen et al. 1981). It is possible that a significant portion of the oxygen required by an individual is taken up across the mantle surfaces and the localisation of mitochondria-rich muscle fibres near the surface maybe related to oxygen uptake (O'Dor et al. 1990). The number of mitochondria-rich muscle fibres and the width of the zone of these muscle fibres may be limited by the diffusion of oxygen across the surface and not related to linear growth of the mantle.

How new muscle fibres arise is poorly understood. Bone et al. (1981) reported observing "small crescentric profiles containing myofilaments" and suggested that these are early muscle fibre precursors. However, this has neither been studied nor observed in further studies. Understanding how new muscle fibres arise will assist in understanding the mechanics of muscle growth and factors affecting muscle fibre recruitment. By understanding the mechanics of growth at the muscle fibre level, it is possible to begin examining how factors such as nutrition, temperature, age, and reproductive status are mirrored in the somatic status of an individual.

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Assessing Smoltification of Juvenile Spring Chinook Salmon (*Oncorhynchus tshawytscha*) Using Changes in Body Morphology

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Beeman, J.W., D.W. Rondorf, and M.E. Tilson. 1994. Assessing smoltification of juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) using changes in body morphology. Can. J. Fish. Aquat. Sci. 51: 836–844.

A morphometric measure of smoltification of juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) was developed and evaluated. Fish were collected from hatcheries in Washington and Idaho prior to release and at McNary Dam on the Columbia River during their downstream migration. Distances between 15 anatomical landmarks were digitized from photographs of each fish resulting in 34 morphometric characters for analysis. The canonical variate calculated from a discriminant function based on several principal components was evaluated as a measure of smoltification. The canonical variate was significantly correlated with gill Na^+–K^+ ATPase activity, a commonly used measure of smoltification. Measuring the morphometric characters and calculating the canonical variate is a relatively simple procedure and can be performed with little harm to the fish. This method of smoltification assessment may be ideally suited to studies in which sacrificing fish is not possible, such as those involving threatened or endangered species, or when access to a laboratory for sample analysis is not available.

On a mis au point et évalué une mesure morphologique de la smoltification de jeunes saumons quinnats (*Oncorhynchus tshawytscha*) du printemps. Des sujets ont été prélevés dans des bassin d'élevage des États de Washington et de l'Idaho avant leur remise en liberté, ainsi qu'au barrage McNary du fleuve Columbia, durant la dévalaison. L'espace entre 15 traits anatomiques a été numérisé à partir de photographies de chaque sujet, ce qui a donné la possibilité d'analyser 34 caractères morphologiques. La variable canonique calculée à partir d'une fonction discriminante établie à partir de plusieurs composantes principales, a été évaluée à titre de mesure de la smoltification. Il existe un rapport significatif entre cette variable et l'activité de la Na^+–K^+ ATPase au niveau des branchies; il s'agit d'une mesure commune de la smoltification. La mesure des caractères morphologiques et le calcul de la variable canonique constituent une méthode relativement simple et qui cause peu de lésions aux poissons. Cette méthode d'évaluation de la smoltification se prête tout à fait aux études où il est impossible de sacrifier des sujets, par exemple parce qu'il s'agit d'espèces en danger ou en voie d'extinction, ou encore parce qu'il est impossible de procéder à des analyses des échantillons en laboratoire.

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Early in the life history of anadromous salmonids, juveniles undergo a metamorphosis, transforming from freshwater-adapted forms (parr) to seawater-adapted forms (smolt). Associated with this process, known as smoltification, are changes in behavior, physiology, and morphology (Hoar 1976). Smoltification of juvenile salmonids has been demonstrated to affect responses to stress, susceptibility to guidance mechanisms at hydroelectric dams, swimming behavior, and migration rates (Smith 1982; Barton et al. 1985; Giorgi et al. 1988; Berggren and Filardo 1993).

Smoltification of juvenile salmonids has been described using many measures of the physiological and endocrinological changes that take place during smoltification development. Common methods include gill Na^+–K^+ ATPase (ATPase) activity, plasma thyroxine (T_4), and seawater challenges (Dickhoff et al. 1985). However, using these methods typically requires either sacrificing the animal to obtain tissue samples or complicated laboratory assays to obtain results. A nonlethal alternative to such methods is a measure of the morphological changes associated with the smoltification of juvenile salmonids (Fessler and Wagner 1969; Gorbman et al. 1982).

Measures of smoltification indicate that juvenile salmonids of hatchery origin undergo smoltification during the in-river migration after release from hatcheries. Measures of smoltification from fish held in captive environments are often low or clearly suppressed compared with migrants (Zaugg et al. 1985; Patino et al. 1986). Gill ATPase activities of migrating juvenile salmonids increase rapidly after release from hatcheries, indicating rapid smoltification (Zaugg 1981; Rodgers et al. 1987). Although some physiological measures of smoltification indicate ongoing smoltification during the in-river migration, little is known about changes in morphology during this time.

Morphology of juvenile salmonids has been used to demonstrate differences among groups of fish with different rearing environments, genetic origins, and life history types (Swain and Holtby 1989; Damsgard 1991; Swain et al. 1991). Winans (1984) recognized the value of this approach and Winans and Nishioka (1987) demonstrated the useful application of this multivariate description of change in

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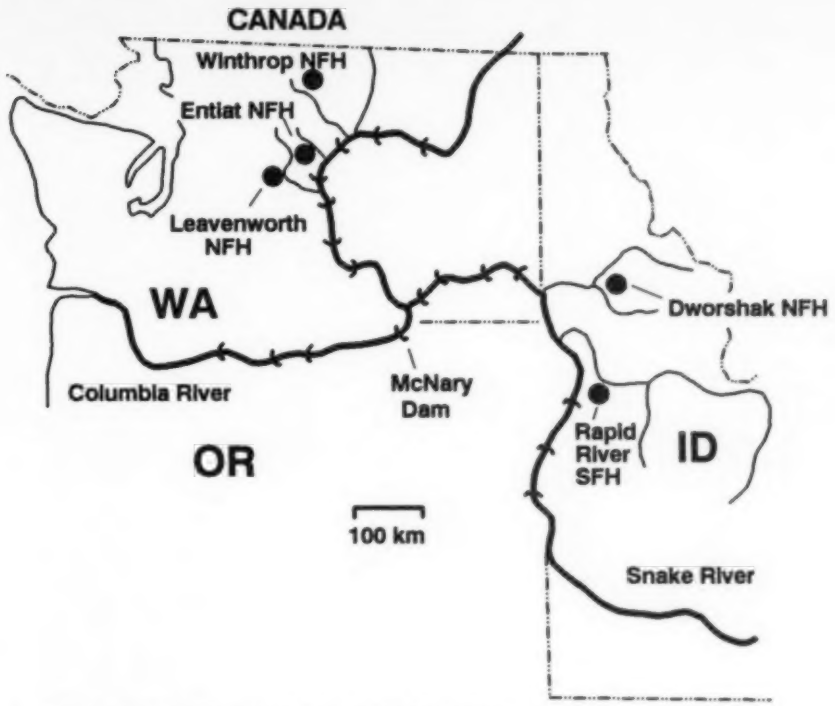

FIG. 1. Map of study area indicating fish hatchery and McNary Dam sample sites.

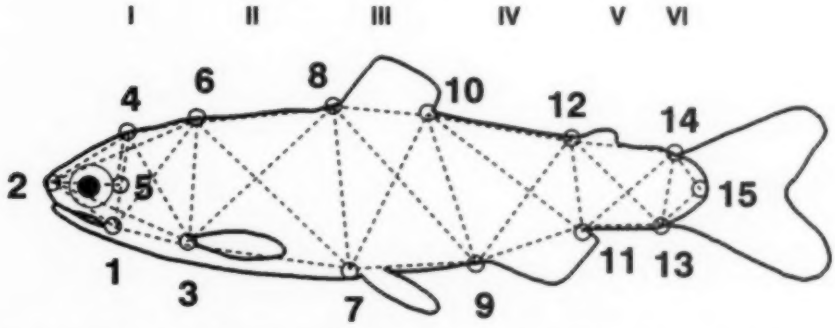

FIG. 2. Locations of 15 landmarks (open circles) and 34 morphometric characters (broken lines) used in the truss network. Landmark points are from Winans (1984) except the locations of (4) the point of the neocranium intersected by the dorsal extension of a line from point 5 perpendicular to character 2–5, (5) the posteriormost aspect of the eye, and (6) the point on the neocranium intersected by a line extended dorsally from point 3 and the posteriormost aspect of the operculum. Roman numerals indicate cells.

body shape during smoltification of coho salmon (*Oncorhynchus kisutch*) in a hatchery environment.

In this paper, we present results from analyses of morphological changes during the seaward migration of juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) of hatchery origin in a large river system. The relations of these morphological changes to other measures of smoltification are described. We propose that a method of assessing smoltification using a nonlethal morphometric index could be substituted for other measures of smoltification.

Material and Methods

This study was conducted on populations of yearling juvenile spring chinook salmon of hatchery origin. The fish were from the Entiat National Fish Hatchery (NFH), Leavenworth NFH, Winthrop NFH (Columbia River hatcheries), Dworshak NFH, and Rapid River State Fish Hatchery (Snake River hatcheries) (Fig. 1). The fish reared at the Columbia River hatcheries originated from the Carson and Little White Salmon river stocks on the lower Columbia

TABLE 1. Loadings from principal components analyses of 34 morphometric characters of 1095 juvenile spring chinook salmon. Loadings are listed for principal component 1 (PC1) and the sheared second (SPC2), third (SPC3), and fourth (SPC4) principal components. Character descriptors and cells refer to Fig. 2.

	Character					
Cell	Number	Descriptor	PC1	SPC2	SPC3	SPC4
I	1	1–2	0.147	−0.060	0.258	0.057
	2	1–3	0.196	0.039	−0.061	0.288
	3	2–3	0.174	−0.011	0.090	0.164
	4	2–4	0.120	−0.026	0.152	0.090
	5	2–5	0.106	−0.013	0.078	0.082
	6	4–5	0.157	0.018	0.127	0.296
	7	4–6	0.240	0.040	0.051	0.410
	8	2–6	0.175	0.003	0.095	0.228
	9	1–6	0.170	0.003	−0.015	0.186
	10	3–4	0.181	−0.026	0.042	0.096
	11	1–4	0.133	−0.032	0.096	0.072
II	12	3–6	0.151	−0.064	−0.058	−0.082
	13	3–7	0.160	−0.042	−0.038	−0.170
	14	6–8	0.154	−0.056	−0.014	−0.235
	15	6–7	0.157	−0.057	−0.049	−0.186
	16	3–8	0.152	−0.056	−0.060	−0.192
III	17	7–8	0.150	−0.081	−0.163	−0.230
	18	7–9	0.185	−0.010	−0.018	0.001
	19	8–10	0.163	−0.050	−0.052	−0.017
	20	8–9	0.172	−0.044	−0.050	−0.087
	21	7–10	0.152	−0.071	−0.164	−0.193
IV	22	9–10	0.173	−0.046	−0.059	−0.123
	23	9–11	0.156	−0.053	−0.053	−0.031
	24	10–12	0.202	−0.029	−0.012	0.105
	25	9–12	0.162	−0.077	−0.100	−0.119
	26	10–11	0.182	−0.022	−0.006	−0.014
V	27	11–12	0.158	−0.033	−0.025	−0.217
	28	11–13	0.241	0.464	0.458	−0.086
	29	12–14	0.169	0.379	−0.218	0.057
	30	12–13	0.192	0.256	0.240	−0.202
	31	11–14	0.195	0.332	−0.272	0.121
VI	32	13–14	0.193	0.230	−0.239	−0.063
	33	14–15	0.188	−0.443	0.463	−0.085
	34	13–15	0.150	−0.397	−0.323	0.290
Variance			0.070	0.013	0.005	0.004
% of total			68.6	13.0	4.5	4.3

River. Fish reared at the Snake River hatcheries were of the Rapid River stock.

The fish were collected at the hatcheries and McNary Dam (river km 470) in spring 1988, 1989, and 1990. In each year, fish bearing freeze brands (Mighell 1969) were collected at each hatchery three times during the 1-mo period prior to release (hatchery residents) and as they migrated past McNary Dam (migrants). Samples collected at the dam were made at approximately the 25th, 50th, and 75th percentiles of each branded group's passage to ensure an even sampling distribution throughout the migration. The juvenile salmon collection system at McNary Dam is equipped with submersible traveling screens to divert juvenile salmonids from the turbine intakes to intake gatewells, where they are bypassed to juvenile fish collection facilities (Matthews et al. 1986).

Sample Methods

Fish sacrificed with a lethal dose of MS-222 were weighed, measured, and photographed immediately after immobilization. Changes in the length and weight relationship were described using Fulton's condition factor (K) calculated for each fish by the equation $(w \cdot l^{-3}) \cdot 10^5$, with weight ($w$) in grams and fork length (l) in millimetres (Ricker 1975). Photographs were taken using a 35-mm camera with black and white film (ASA 100) mounted on a copy stand. Fish were photographed with the fins held in the extended position using pins, using care to avoid altering the body shape, and a millimetre scale was placed nearby.

Morphometric data were collected from photographs by digitizing anatomical landmarks in an X and Y coordinate system using a digitizer and personal computer. Morphometric characters, forming a truss network similar to that in Winans (1984), except as noted in Fig. 2, were calculated from the X and Y coordinates. Distances between 15 landmark locations were calculated, resulting in 34 morphometric characters for analysis (Fig. 2). These characters were adjusted using the scale included in each photograph so that each digitized distance corresponded to the actual distance on the fish. To simplify interpretation, the morpho-

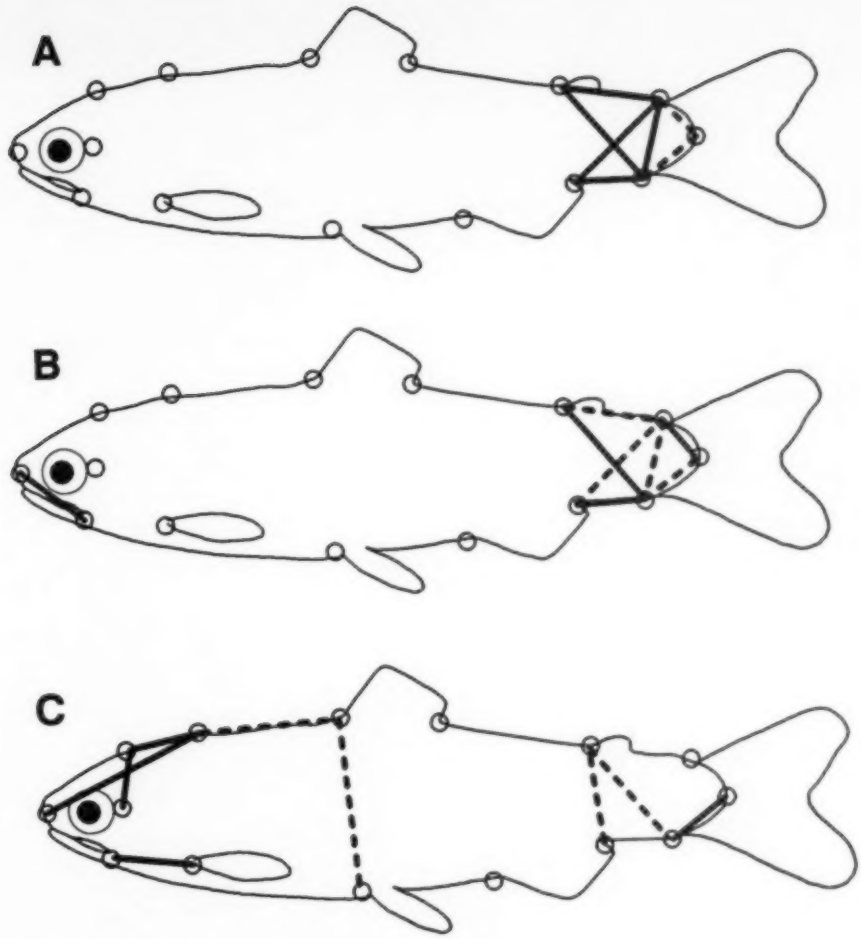

FIG. 3. Schematic diagrams of morphometric characters with important loadings on (A) SPC2, (B) SPC3, and (C) SPC4. Solid lines denote characters with positive loadings; broken lines denote those with negative loadings.

metric characters were grouped into six cells along the anterior–posterior axis of the fish (Fig. 2).

Gill filaments and blood plasma were collected to assess smoltification through measurements of gill ATPase activity and plasma T_4. Gill filaments removed from the left gill arches were preserved and assayed using the method of Zaugg (1982). Blood plasma samples were assayed for plasma T_4 using the radioimmunoassay described by Dickhoff et al. (1978) and modified by Specker and Schreck (1982).

Principal Components Analysis

The variance–covariance matrix of the log-transformed (base 10) set of 34 morphometric characters was analyzed using principal components analysis. Principal components analysis was used as a data reduction procedure to reduce the large data set of morphological traits to several principal components for further analysis (Tabachnick and Fidell 1983).

To permit analyses of shape without the effects of allometric growth, a size-adjustement procedure was necessary because fish in the migrant group had a significantly larger mean fork length than those in the hatchery-resident group. The second (PC2), third (PC3), and fourth (PC4) principal components were sheared to adjust for the effects of size using the shearing method of Humphries et al. (1981) as reformulated by Rohlf and Bookstein (1987). In this method the first principal component is used as a latent factor to represent general size. A computer program from Bookstein et al. (1985) and modified by Rohlf and Bookstein (1987) was used for this purpose after modification for use with a personal computer. Data analysis was performed using the SAS language for personal computers (SAS Institute 1989).

The correlations of sheared principal component scores with other measures of smoltification were analyzed using the Pearson product-moment correlation. In this analysis the number of days of in-river migration (MIGDAYS), calculated as the number of days between the date of release from the hatchery and date of recapture at McNary Dam, was used as a time variable to examine correlations of other variables with time-related migratory development. Correlations between individual scores of sheared PC2 (SPC2), sheared PC3 (SPC3), and sheared PC4 (SPC4) with

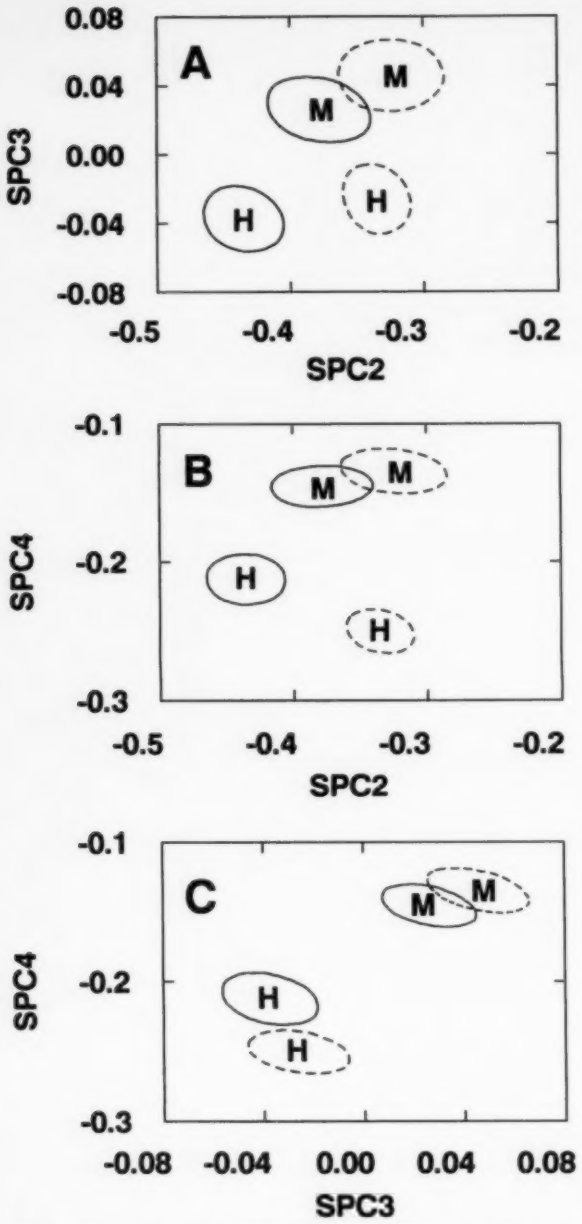

FIG. 4. Bivariate plots of SPC2, SPC3, and SPC4 with 95% confidence ellipsoids around bivariate means from juvenile spring chinook salmon from Columbia River hatcheries (solid lines) and Snake River hatcheries (broken lines). Sample groups: H = hatchery residents, M = migrants.

MIGDAYS, *K*, gill ATPase activity, and plasma T_4 concentrations were examined.

Discriminant Function

Canonical discriminant function analysis was used to classify fish in the training data set (n = 1095) as either hatchery residents or migrants based on SPC2, SPC3, and SPC4. This data set included 485 hatchery residents and 610 migrants collected in 1989 and 1990. Homogeneity of the within-group variance–covariance matrices was tested using Bartlett's modification of the likelihood ratio test to determine whether a linear or quadratic discriminant function was appropriate (SAS Institute 1989).

Two methods were used to evaluate the performance of the discriminant function. In the first, a cross-validation procedure was used to determine if the classification error-rate estimates of the discriminant function were biased. This procedure involved deleting an observation from the training data set, estimating a discriminant function based on those remaining, and classifying the deleted observation as to group membership. This procedure was repeated for each observation in the data set. This approach is similar to a jack-knife procedure (Efron 1982) and allows all of the fish in the training data set to be used for validation.

As a second method of verification, the percent correct classification of fish independent of those used to develop the discriminant function was examined. In this method, fish collected from the hatcheries and McNary Dam in 1988 (n = 1022) were classified using the discriminant function based on the training data set. This method was also used as a measure of the effectiveness of the discriminant function in classifying fish from other years.

The canonical variate (CAN) was evaluated as a morphological index of smoltification. Pearson product-moment correlations were used to measure the association of CAN with gill ATPase activity and plasma T_4 of fish in the training data set. Statistical results were considered significant when $P \leq 0.05$.

The sample size required to detect a difference of 0.5 unit between two mean CAN values was calculated as an estimate of the sample size required when using the morphometric measure of smoltification. This number was estimated based on a 90% chance ($\beta = 0.10$) of detecting a true difference between two mean CAN values at the $\alpha = 0.05$ significance level (Zar 1984). Variability in the data was based on the sample variances of CAN values of the hatchery residents and migrants in the training data set.

Results

Principal Components Analysis

Ninety percent of the variation of the 34 morphometric characters was accounted for using the first four principal components. Variable loadings of the first component (PC1), explaining 68.6% of the total variation, were of similar size and sign, indicating that PC1 was a general size component (Pimentel 1979; Bookstein et al. 1985) (Table 1). This component was highly correlated with fork length (r = 0.97, n = 1092, P = 0.0001). The SPC2, SPC3 and SPC4 explained 13.0, 4.5, and 4.3% of the total variation, respectively. Loading of these components were of variable sizes and signs and were interpreted as shape components. Each of the remaining principal components accounted for less than 2% of the total variation; these were not examined further.

The SPC2 was characterized by large positive loadings in cell V and large negative loadings in cell VI; it summarized variation associated with caudal peduncle shape (Table 1; Fig. 3). Variation described by this component was predominantly due to differences among Columbia and Snake River fish, although some differences between hatch-

TABLE 2. Correlation coefficients, significance levels (in parentheses), and sample sizes for comparisons between several variables measured in juvenile spring chinook salmon collected in 1989 and 1990 and included in the training data set used in the canonical discriminant function. Refer to text for variable definitions.

	ATPase	SPC2	SCP3	SPC4	T_4	*K*
MIGDAYS	0.8625	0.2950	0.4447	0.6147	−0.0336	−0.8189
	(0.0001)	(0.0001)	(0.0001)	(0.0001)	(0.3995)	(0.0001)
	1095	1095	1095	1095	629	946
ATPase		0.2912	0.3967	0.6371	−0.0719	−0.7130
		(0.0001)	(0.0001)	(0.0001)	(0.0715)	(0.0001)
		1095	1095	1095	629	946
SPC2			0.0260	0.0379	−0.0141	−0.1386
			(0.3899)	(0.2102)	(0.7239)	(0.0001)
			1095	1095	629	946
SPC3				0.0496	0.0108	−0.5579
				(0.1012)	(0.7879)	(0.0001)
				1095	629	946
SPC4					−0.0547	−0.5560
					(0.1707)	(0.0001)
					629	946
T_4						−0.0333
						(0.4332)
						555

ery residents and migrants from the Columbia River hatcheries were also evident; those from the Snake River hatcheries were poorly separated using SPC2 (Fig. 4A and 4B). This component indicated that fish from the Columbia River hatcheries had generally longer (cell V), but more posteriorly truncated (cell VI), caudal peduncles than those from the Snake River hatcheries. In addition, the mean SPC2 of migrants from the Columbia River hatcheries was higher than those of hatchery residents, indicating that SPC2 increased during the migration of these fish.

The most important loadings of SPC3 were generally characterized by a contrast of several positive and negative values in cells V and VI, as well as one positive loading in cell I; it summarized variation associated with caudal peduncle shape apart from that described by SPC2 (Table 1; Fig. 3). This component accounted for variation between hatchery residents and migrants of both drainages (Fig. 4A and 4C).

The most important loadings of SPC4 were characterized by a contrast of several positive loadings in cell I and one positive loading in cell VI, with many, predominantly negative, loadings in all other cells (Table 1; Fig. 3). This component indicated that migrants from Columbia and Snake River hatcheries had larger heads and smaller bodies compared with hatchery residents (Fig. 4B and 4C).

Individual SPC2, SPC3, and SPC4 scores were significantly correlated with gill ATPase activity, *K*, and MIGDAYS (Table 2). The significant correlations with MIGDAYS indicated that the changes in shape were associated with the time elapsed during the migration. The correlations between the principal components and gill ATPase activity were interpreted as evidence that they described shape changes associated with smoltification. The principal components were positively correlated with gill ATPase activity; they increased as smoltification development progressed during the migration. Principal components SPC2, SPC3, and SPC4 were not correlated with plasma T_4 concentrations ($P > 0.1$).

Discriminant Function

Ninety-two percent of fish in the hatchery-resident and migrant groups in the training data set were correctly classified into group of origin using SPC2, SPC3, and SPC4 as variables in a quadratic discriminant function (Table 3). A quadratic discriminant function was selected because the within-group variance–covariance matrices were significantly different (chi-square = 65.7, $P = 0.0001$). Cross-validation error rates were identical to those in the original classification, indicating the error rate estimates from the training data set were unbiased.

TABLE 3. Classification results of discriminant function analyses to separate hatchery resident (H) and migrant (M) groups of juvenile spring chinook salmon.

Actual group	Number of fish classified as: H	M	Total	% correct
	Training set			
H	447	38	485	92
M	50	560	610	92
	Cross-validation set			
H	447	38	485	92
M	50	560	610	92
	Independent set			
H	490	122	612	80
M	24	386	410	94

Eighty percent of hatchery residents and 94% of the migrants independent of those used to develop the discriminant function were correctly classified. This indicated the results that could be expected when the discriminant function is applied to other independent samples, and that the discriminant function can be successfully applied to fish collected in different years. The canonical discriminant function equation was

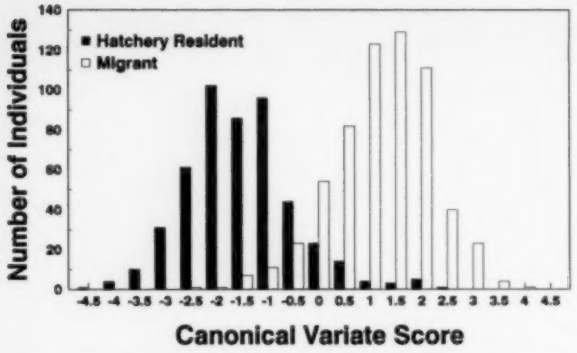

FIG. 5. Distribution of CAN scores among hatchery residents (solid bars) and migrants (open bars) in the training data set.

$$\text{(1)}\quad \text{CAN} = 5.059 + 3.294\ (\text{SPC2}) + 13.000\ (\text{SPC3}) + 18.976\ (\text{SPC4})$$

where the sheared principal components are calculated as

$$\text{(2)}\quad \text{SPC}_j = \sum_{i=1}^{34} (\log_{10} l_i \cdot L_{ij})$$

where i designates the character number from 1 to 34, l_i is the length of character i in millimetres, and L_{ij} is the loading of character i on the jth principal component.

Fish with a CAN score less than zero were classified as hatchery residents and those with a score above zero as migrants (Fig. 5). CAN scores were significantly correlated with gill ATPase activity ($r = 0.77$, $n = 1095$, $P = 0.0001$) but not with plasma T_4 ($n = 629$, $P = 0.2994$).

The relations between MIGDAYS, CAN, and gill ATPase activity of two representative groups collected during 1990 are illustrated in Fig. 6. In each instance, the trends in CAN are similar to trends in gill ATPase activity over time. Values of both variables were lower in hatchery residents (MIGDAYS ≤ 0) than in migrants (MIGDAYS > 0), with values increasing sharply after release from the hatcheries. The variability of CAN was higher than that of gill ATPase activity, indicated by the larger standard errors in Fig. 6.

The standard deviation of CAN of the hatchery residents in the training data set (SD = 1.04) was used to estimate the sample size required rather than the slightly smaller standard deviation of CAN of the migrants (SD = 0.97). Based on this conservative approach, the sample size required to detect a difference of 0.5 unit between two mean CAN values was estimated as $n = 92$.

Discussion

Morphological changes occur in juvenile spring chinook salmon during smoltification, and these changes can be quantified. The SPC2, SPC3, and SPC4 indicated that shape changes occurred in the caudal peduncle, head, and body depth, with differential growth in these areas resulting in migrants with longer caudal peduncles, larger heads, and narrower bodies than hatchery residents. The largest amount of shape variability, accounted for by SPC2, was from landmarks 11–13 in the caudal peduncle (Table 1). These results are similar to those described during smoltification of hatchery-resident juvenile spring chinook salmon and coho

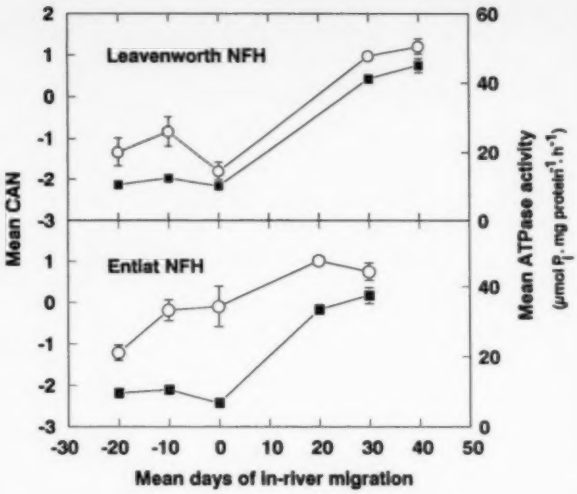

FIG. 6. Plot of mean Na^+–K^+ gill ATPase activity (squares) and the mean canonical variate (circles) versus mean days of in-river migration of two representative groups of hatchery spring chinook salmon collected in spring 1990. Sample sizes range from 10 to 28; vertical bars indicate ±1 SE.

salmon (Winans 1984; Winans and Nishioka 1987) and anadromous and resident Arctic char (*Salvelinus alpinus*) (Damsgard 1991).

The timing of shape changes occurring during smoltification of the groups we examined was similar to the development of gill ATPase activity. Most changes in shape occurred after release from the hatchery environment, proceeded rapidly for several weeks, and then slowed. However, our data were limited to the time between 1 mo prior to release (March and April) and 60 d afterward; there may also be changes in shape prior to or after this time. Winans and Nishioka (1987) reported cyclical changes in the caudal peduncle shape of juvenile coho salmon in a California hatchery over a several-month period.

The shape variability accounted for by each of SPC2, SPC3, and SPC4 was necessary to develop a canonical variate with optimal discriminatory power and the highest correlation with gill ATPase activity. Although most shape variation in the training data was accounted for by SPC2, hatchery residents and migrants from the Snake River hatcheries were poorly separated along this axis, requiring the addition of the other principal components to the discriminant function. The SPC3 and SPC4 variables explained small amounts of the total variability in the training data set, but were the primary axes along which hatchery residents and migrants were separated. The discriminant function analysis was used to weight each of these principal components based on their relative discriminatory powers, resulting in low classification error rates and a high correlation with gill ATPase activity.

The sample sizes required to result in precise estimates of CAN may vary depending on the application. The determination of appropriate sample sizes depends not only on the level of precision required, but also on the variability of the data collected. Our sample size estimate was based on detecting a small difference in CAN and on the variability

associated with a composite sample of hatchery residents from five hatcheries. We believe it is a conservative estimate which is probably an upper bound of the sample size required for most applications. Smaller sample sizes may be appropriate if the acceptable level of precision is lower. For example, an estimated sample size of $n = 23$ is required to detect a difference of 1.0 CAN unit. However, since changes in shape occur rapidly during the first several weeks of migration, morphological data from fish with varied migration histories collected during this time may require larger sample sizes. This may occur when morphometric data are collected from migrating fish of many different or unknown origins. The appropriate sample sizes for a specific application may be best estimated based on previous data from the application.

Other nonlethal measures of smoltification are available, such as the gill ATPase assay of McCormick (1993), which requires only a small gill biopsy. However, this method requires a laboratory with specialized equipment and technicians which may be unavailable to many researchers and managers.

The morphometric measure of smoltification has the advantage of being nonlethal as well as requiring little training and having a relatively fast turn-around time from sample collection to final result. With practice, fish can be digitized at a rate of about 2 min each, requiring approximately 3 h to digitize a sample of $n = 90$ fish. Using a simple computer program, results can be available as the fish are digitized. Moreover, recent developments in video technology may make it possible for images to be digitized when they are collected, rather than digitizing from still photographs. However, the morphometric methods to assess smoltification may be less desirable than others in situations when samples need to be collected in a short amount of time, or when sufficient space is not available, conditions common to many juvenile fish traps.

Using a morphometric indicator as a measure of smoltification provides researchers and managers with a tool that is nonlethal to fish, requires a reasonable sample size, has relatively low equipment costs, and requires little technical training compared with laboratory assays. The morphometric method of smoltification assessment described here may be best suited to studies in which sacrificing fish is not possible, such as those involving threatened or endangered species, or when access to a laboratory for sample analysis is not available.

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ARROW Oil Spill, 1970–90: Persistence of 20-yr Weathered Bunker C Fuel Oil

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Vandermeulen, J.H., and J.G. Singh. 1994. *ARROW* oil spill, 1970–90: persistence of 20-yr weathered Bunker C fuel oil. Can. J. Fish. Aquat. Sci. 51: 845–855.

A full range of weathered petroleum residues persist in two beach sites in Chedabucto Bay, Nova Scotia, 20 yr after the *ARROW* Bunker C spill. Both sites contained *ARROW* bunker fuel residues (pentacyclic triterpane characterization). Residues from "tar pavement" and a tar deposit within a cobble spit retained many components of original *ARROW* Bunker C fuel. Results indicate that long-term persistence is a direct function of beach sediment permeability, and of the depth to which entrapped tar residues penetrate; persistence is an inverse function of the frequency and depth of sediment reworking during tidal incursions. Cobble beaches represent an extreme example, with the interstices acting as low-energy sinks for spilled oil. A revised model for oil stranding and fate proposes two distinct environmental reservoirs of stranded oil residue in these medium-grained beaches: the interstitual residue as the major long-term source and surface-stranded residue as the minor source, each with its own weathering rate, history, and persistence. Such in-beach sequestered oils undergo much slower weathering than surface-stranded residues, persist longer, and continue to be remobilized. However, actual transfer of hydrocarbons from such deposits to tidal waters is very low (ppb).

Une gamme complète de résidue de goudron altérés par l'environnement persiste dans deux sites de pollution par pétrole à Chedabucto Bay, Nouvelle-Écosse, 20 ans après la marée noire du *ARROW* Bunker C. Tous contenaient des résidus identifiables de fuel provenant du *ARROW* Bunker C (confirmé par caractérisation au triterpane pentacyclique). Certains résidus provenant de couverture de goudron et d'une dique en galets étaient virtuellement identiques au fuel original de l'*ARROW* Bunker C. Les mares contenaient tous des résidus de Bunker fuel. Les résultats indiquent que la présence à long term est directement fonction de la perméabilité des sédiments de plage, de la profondeur à laquelle les résidus de goudron piégés pénètrent, et est une fonction inverse de la fréquence et de lépaisseur du brassage du sédiment au cours du cycle de marée. Les plages de galets représentent un exemple extrême, les interstices agissan comme des puits de basse énergie pour le pétrole déversé. Un modèle d'échouage du pétrole, basé sur la caractérisation des résidus de pétrole par chime analytique, suggère que les résidus d'hydrocarbures contenus dans les plages dérivent probablement de déversements de pétrole qui pénètrent les sédiments des plages au moment de l'échouage, et ne dérivent pas, comme suggéré précédemment, de résidus de surface échoués le long des lignes de haute mer. Les produits pétroliers séquestrés dans les plages subissent une altération beaucoup plus lente, avec des temps de présence plus long que les résidues échoués de surface.

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This paper addresses the long-term persistence of petroleum residues, remaining on and in beach sediments 20 yr after the *ARROW* oilspill in 1970 in Chedabucto Bay, Nova Scotia, Canada. At that time, an estimated 2 million L of Bunker C fuel oil was stranded ashore (Ministry of Transport 1970a). Much of the approximately 200 km of oiled shoreline was left to natural cleaning processes such as tidal and wave washing, sediment scouring, microbial degradation, and evaporation. These processes proved successful for degrading much of the visible surface tar. Subsequent surveys indicated a half-life for self-cleaning of stranded surface oil of 2–3 yr in mixed sediments in this north-temperate climate (Rashid 1974; Owens and Rashid 1976; Vandermeulen 1977). This agrees with more recent estimates of 6 mo for sandy or gravel beaches and about 3 yr for mudflats (Reed et al. 1989). However, residues of stranded oil can persist for much longer periods. For example, Teal et al. (1992) recently reported on No. 2 fuel hydrocarbons, still detectable in salt marsh sediments, 20 yr after a fuel oil spill from the barge *FLORIDA*. Also, Owens et al. (1993) described Bunker C residues in a number of locations in Chedabucto Bay, site of the 1970 *ARROW* spill. Commonly, such long-term residues are generally heavily weathered and have little chemical resemblance to the original spilled oil, with most of the volatile and biodegradable components having been lost. Indications from field observations carried out by ourselves, however, suggest that some long-term stranded residues can in fact undergo relatively little weathering under certain conditions, even within high-energy cobble beaches. It is this aspect of the fate of spilled oil in coastal zones that interests us here.

We have revisited two locations that were heavily oiled following the *ARROW* oil spill (Ministry of Transport 1970a, 1970b, 1970c) that still contain traces of oil residue (personal observation; Owens et al. 1993). They differ in oceanic

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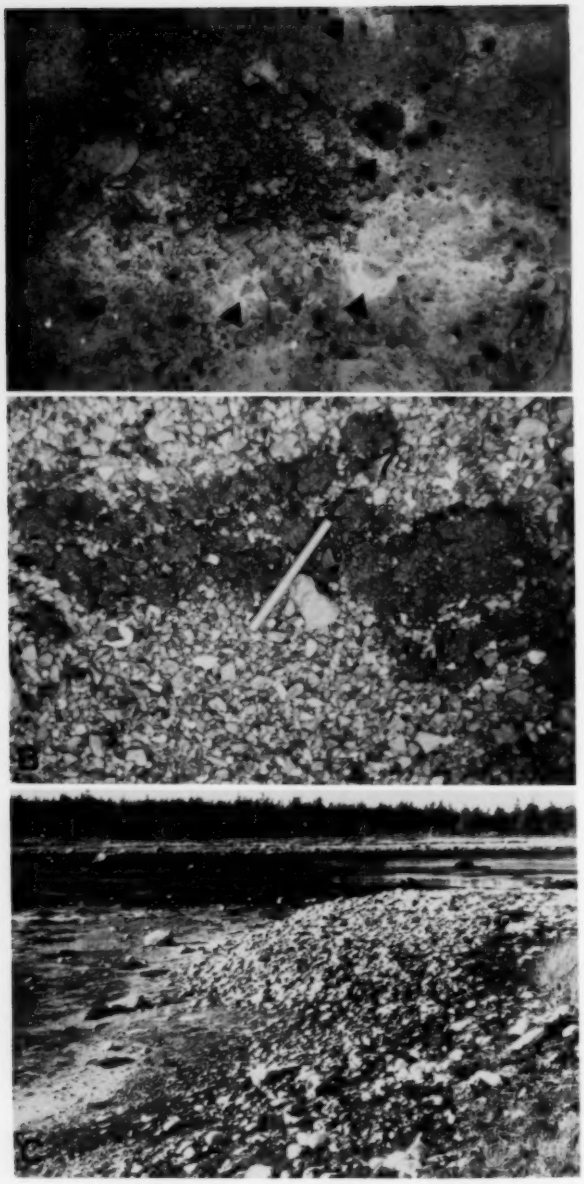

FIG. 1. Twenty-year-weathered stranded *ARROW* Bunker C residues in Chedabucto Bay, N.S. (A) Oil sheen (arrowheads) on Janvrin Lagoon beach sediment; (B) weathered Bunker C asphalt, Black Duck Cove; (C) consolidated cobble–tar pavement (foreground), Black Duck Cove.

exposure. Janvrin Lagoon is a small, low-energy lagoon located within Chedabucto Bay. Its shoreline consists mainly of a 3- to 10-cm layer of poorly sorted silt, sand, and gravel overlying a clay basement. It is protected from the open bay by a long slender cobble–boulder spit and supports an abundant eelgrass (*Zostera marina*) and soft-shell clam (*Mya arenaria*) community (Gilfillan and Vandermeulen 1978; Thomas 1978) in a largely muddy bottom. At the time of this study, oil sheens were visible on the beach surface at low tide along much of the shoreline (Fig. 1A) and in water welling up in clam diggings. Dried tar flakes still marked the high tide line along some parts of the shore.

Black Duck Cove is a small, medium- to high-energy cove located on the outer North Atlantic coast, a few kilometres south of Chedabucto Bay. It is a rocky cove and sandy beach system, protected from the Atlantic Ocean only by a broad cobble–boulder spit. An inner sand flat supports a population of sandworms (*Arenicola marina*) (Gordon et al. 1978). Tarry residue is evident in much of the intertidal zone (Fig. 1B); a pavement of Bunker C fuel oil that had consolidated in an intertidal cobble–boulder area remains virtually intact (Fig. 1C). It had been left to "stabilize" at the time of the spill, until it could be cleaned more easily.

Both locations had been left to recover naturally (self-clean) (F. Barber, personal communication; Ministry of Transport 1970b), but today, both still contain residues of oil and tar. For our purpose, the sites offered these experimental advantages: (1) uniform oiling at the time of the *ARROW* accident and (2) a continuous monitoring record (Owens 1971; Thomas 1971; 1973; Rashid 1974; Owens and Rashid 1976; Gilfillan and Vandermeulen 1978; Gordon et al. 1978; Keizer et al. 1978; Humphrey and Vandermeulen 1986; Owens et al. 1993). Particular attention was given to confirming the *ARROW* Bunker C origins in these environmental samples, including by pentacyclic triterpane "fingerprinting".

Materials and Methods

Sampling

For Janvrin Lagoon, we collected heavily weathered asphaltic "stranded beach tar" and "heavily oiled sediment" from an intertidal area in which the sediment visibly contained oily residue. We also included some specimens of soft-shell clams (*Mya arenaria*) and mussels (*Mytilus edilus*). Samples collected in Black Duck Cove included outer "crust tar" of tar pavement, "tar" taken from within tar pavement, "water over tar" pavement taken at flood tide, "intertidal water" collected from a large depression in tar pavement, near-flowing "spit tar" oozing from boulders within the boulder spit, and "weathered oil residue" smears scraped from the face of a boulder in the high-energy zone of the spit.

All tar and sediment samples were stored in solvent-rinsed containers. Original *ARROW* Bunker fuel, continuously stored under nitrogen, served for analytical comparison with the weathered material.

Analytical Methodology

Raw tar samples (weathered tar flecks, asphaltic tar, fluid spit tar) were dissolved in dichloromethane, filtered, and resuspended in hexane. This was followed by fractionating into aliphatic (F1) and aromatic (F2) fractions using alumina/silica gel adsorption column chromatography (MacLeod et al. 1988). Fractions were concentrated by Kuderna-Danish evaporation. Hydrocarbons in water samples were first partitioned into dichloromethane and then into hexane (Keizer and Gordon 1973). Hydrocarbons in sediment, clam, and mussel soft tissues were extracted by alkaline saponification (Obana et al. 1981; Donkin and Evans 1984; Lawrence and Weber 1984) and then treated as above.

UV fluorescence (UV/F) was used for routine quantitative

TABLE 1. Composition of 20-yr shoreline tar residues and percentage hydrocarbon composition in Janvrin Lagoon and Black Duck Cove.

Tar residue	Ratio of tar to debris[a]	Percentage hydrocarbon fraction[b]		
		%F1	%F2	%uneluted
ARROW Bunker C	99:1	41.8	39.6	58.2
Janvrin Lagoon				
Stranded asphalt	6:94	12.6	26.7	60.7
Oiled beach sediment[c]	0.04:99.8	9.7	38.2	52.1
Black Duck Cove				
Tar smear	31:69	22.2	29.9	47.9
Tar pavement	12:88	42.2	32.8	25.0
Fluid spit tar	25:75	38.4	39.9	21.7

[a]Gravimetric determination from triplicate samples after dichloromethane extraction.
[b]GC/FID determination; "Standard methods for the examination of water and wastewater, 1975, p. 515–516.
[c]Extraction by alkaline saponification (Obana et al. 1981).

determinations (fixed excitation-emission 310–365 nm), and qualitatively in the synchronous ($\Delta\lambda$ = 20 nm) excitation–emission scan (SEES) mode (Hargrave and Phillips 1975; Frank and Pernell 1976; Bidleman et al. 1990). The SEES peak height at excitation = 337 nm, emission = 357 nm was also used quantitatively, with whole *ARROW* bunker C fuel oil as the standard or reference material. In comparison, SEES was found to be more sensitive than the more commonly used fixed excitation–emission technique.

Gas chromatography (GC) (Hewlett Packard 5730A gas chromatograph, FID) was used qualitatively and quantitatively (pristane and phytane ratios, UCM (unresolved complex mixture) boiling range) to determine weathering in environmental samples and to discriminate between material of biogenic and petrogenic origin (Rowland and Volkman 1982; Shaw et al. 1985; Dahlmann 1987). All F2 fractions, with the exception of the very heavily weathered material, were analyzed qualitatively for 16 polycyclic aromatic hydrocarbons (PAHs) by GC coupled with mass selective detector (MS) (HP 5890A GC, model 5970B MSD, UNIX data system; splitless injection, 20-m DB-5 capillary column, 0.18-mm inside diameter, temperature programmed from 45 to 280°C at 10°C/min after an initial 2-min isothermal period, final temperature held for 30 min).

GC-coupled mass spectroscopy (GC/MS) was used to match tar source by the presence of characteristic pentacyclic triterpanes. The presence of (17αH,21βH)-hopane-type triterpanes and 22*R*- and 22*S*-diastereomer has been suggested as a definitive indicator of petroleum and petroleum contamination (Dastillung and Albrecht 1976; Seifert and Moldowan 1980; Grahl-Nielsen and Lygre 1990). Selected F1 fractions were analyzed on the Hewlett Packard 5890 GC/MS system operating in the selected ion monitoring mode (SIM) (*m/e* 191.3) (helium, temperature program 75–280°C at 10°C/min after an initial 2-min isothermal period, final temperature held for 30 min). Peaks were identified from available files. Fourteen major peaks, and other minor peaks that assume prominence in some oils, established as triterpanes by GC/MS (Pym et al. 1975; Gilbert 1987), were selected from the resolved peaks present in each chromatrogram. Each of the 14 peaks (A–J) was integrated and its areas normalized to the aggregate area of the 14 peaks. Duplicate subsamples were analyzed and mean normalized areas calculated.

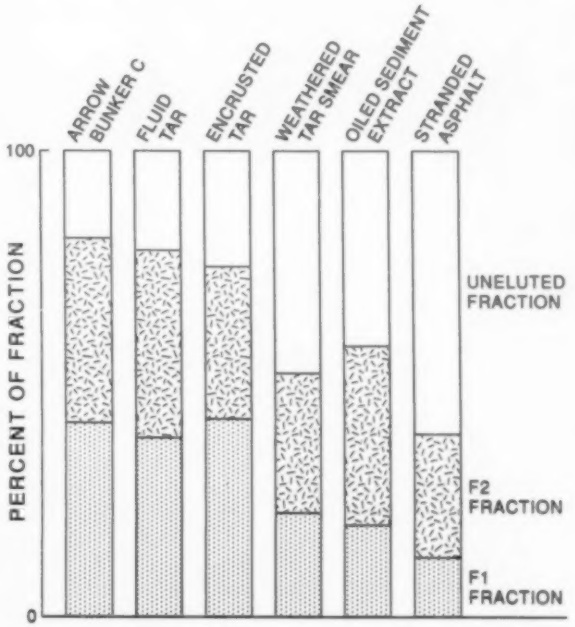

FIG. 2. Relative composition of tar residues from Chedabucto Bay in terms of aliphatic (F1), aromatic (F2) and uneluted asphaltene fractions. Gravimetric determinations.

Results

Chemical Composition

Gravimetric and GC/FID results (Table 1) indicate three general findings. (1) All petroleum residues contained nonpetrogenic material (beach detritus, sand or gravel, organic matter), varying from 68% in weathered tar smears to 99.8% in oiled beach sediments. Also, all residues contained varying amounts of noneluted and unresolved high molecular weight hydrocarbon material (asphaltenes) (Fig. 2). (2) As expected for 20-yr-old fuel oil remains, residues showed various stages of weathering (i.e., alteration by erosion, dissolution into seawater, evaporation, photodegradation, biological and microbial degradation). For example, stranded

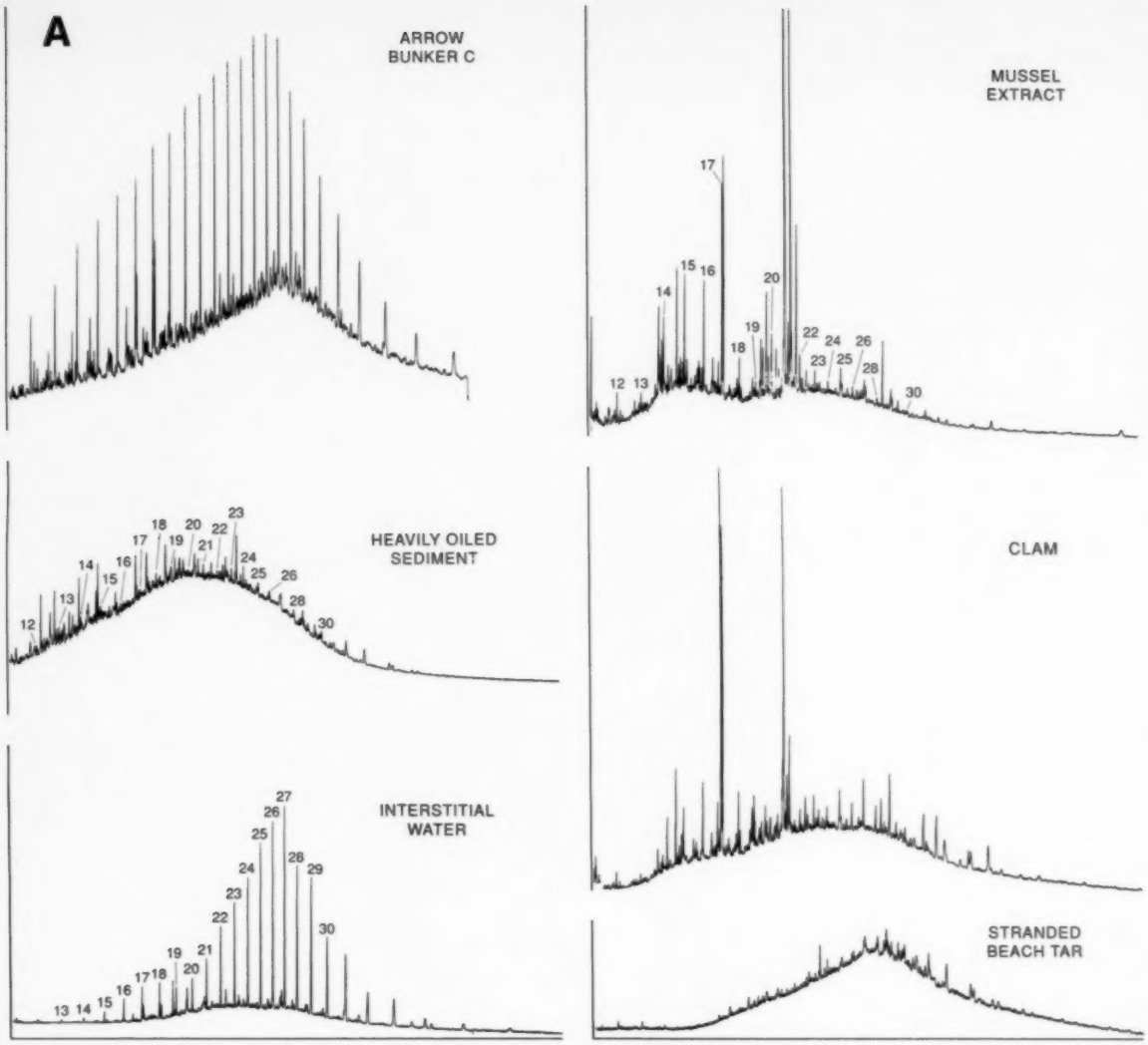

FIG. 3. Gas chromatograms (GC/FID) of F1 aliphatic components in 20-yr-weathered environmental samples from (A) Janvrin Lagoon and (B) Black Duck Cove. (*Fig. 3. continued on next page*)

beach asphalt from Janvrin Lagoon and a sample of tar smear from Black Duck Cove had both lost about two thirds of their aliphatic fraction and up to one third of their aromatic components (Fig. 2). (3) Unexpectedly, two Black Duck Cove samples ("subcrust tar", fluid spit tar) superficially appeared unweathered, although there was some increase in the unresolved material.

GC/FID analyses (Fig. 3) provide qualitative support for the gravimetric results and also provide evidence of biodegradation and of input from biogenic sources. Comparison of the chromatograms shows the typical stages of weathering of petroleum hydrocarbons including (a) loss of low-boiling aromatic and saturated hydrocarbons (e.g., pavement crust tar, weathered oil residue) and (b) increasing size of the UCM. Concentrations of resolved hydrocarbons in these 20-yr-old residues varied widely between exposed and protected locations, most obviously in the F1 chromatograms (Fig. 3). For example, weathered oil residue (i.e., tar smears) is marked by the absence of <C20 *n*-alkanes and significant reduction of >C20 resolved components, as well as an enhanced UCM. Stranded beach tar (Janvrin Lagoon) exemplifies extreme weathering (evaporation, dissolution, microbial degradation) with total loss of resolved *n*-alkanes. Water samples collected from over tar pavement show similarly weathered hydrocarbon profiles; the persistence of >C20 *n*-alkanes, albeit in decreased levels, is typical of weathering by evaporation and/or dissolution. In contrast, protected tar residues still retain some or much of the *ARROW* Bunker C suite of *n*-alkanes, albeit in conjunction with a sizable UCM, e.g., spit tar.

The contrast between exposed and protected weathering states is most evident in the tar pavement samples from Black Duck Cove (Fig. 3B). The exposed tar crust is virtually absent of resolved material, showing mainly a sub-

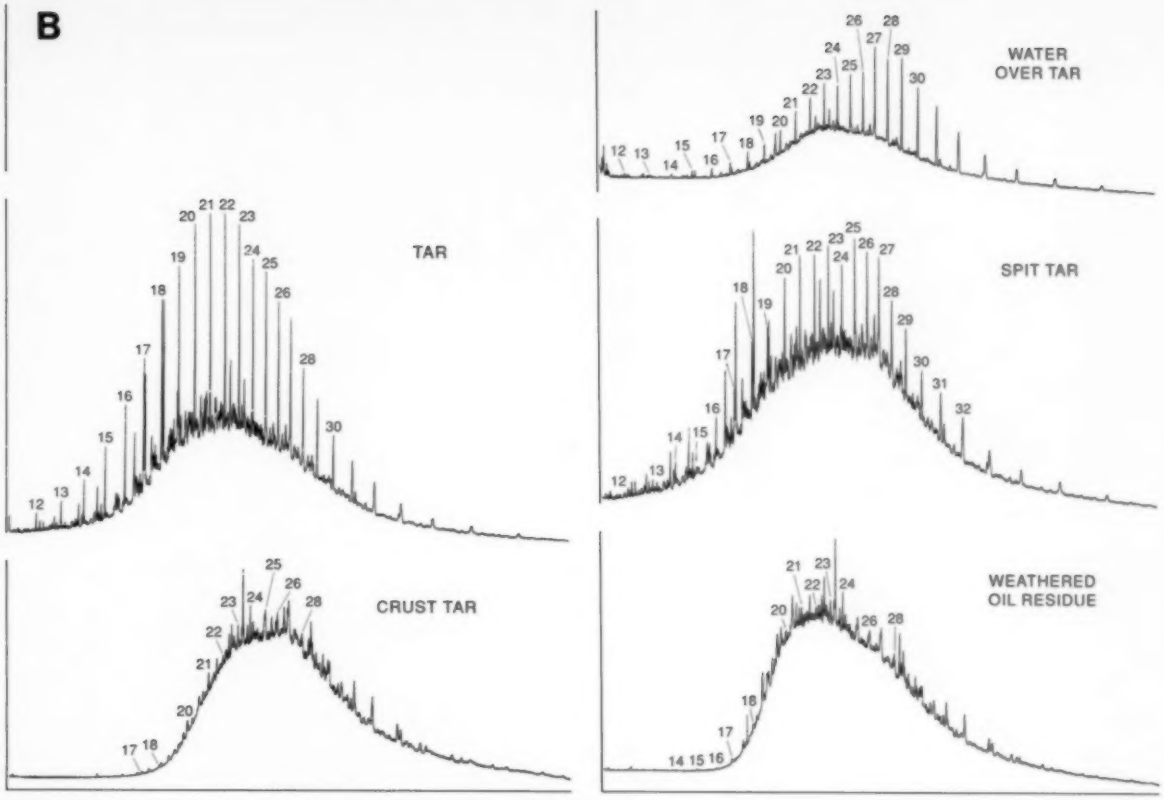

FIG. 3. (*Concluded*)

TABLE 2. Pristane to phytane ratios and UCM boiling ranges in aliphatic fractions of tar residues in Chedabucto Bay (determined by GC/FID). n.d. = one or both peaks not detected. Terminology in quotations in Fig. 3.

Sample	Pristane:phytane	UCM boiling range
ARROW Bunker C	0.81	C10–C3o
Black Duck Cove		
Fluid spit tar	0.74	C12–C36
Tar pavement, interior tar	0.69	C13–C36
Tar pavement, crust tar	n.d.	C17–C36
Water over tar	0.71	C17–C36
Intertidal water	0.69	C17–C33
Weathered oil residue (tar smear)	n.d.	C16–C36
Janvrin Lagoon		
Heavily oiled sediment	n.d.	C11–C36
Stranded beach tar	n.d.	C16–C36
Unoiled beach sediment	3.25	C14–C36

stantial UCM, and closely resembling the F1 chromatogram of tar smears. This is not unexpected because of its continuous exposure to sun, wave action, tidal washing, sediment scouring, and evaporation. In contrast, tar sampled from within the tar pavement has retained a well-maintained *n*-alkane profile similar to that of original Bunker C (Fig. 3). Even so, a much increased UCM, plus the shift of the UCM boiling range with respect to that in original *ARROW* material, indicates considerable weathering (Fig. 3; Table 2).

Pristane to phytane ratios in several of the Janvrin Lagoon samples and in control mussel samples (Table 2) generally are greater than unity, suggesting strong contribution from biogenic sources (NRC 1985, p. 31). The better preserved tar residues from Black Duck Cove and the *ARROW* oil sample show pristane to phytane ratios <1.00.

A broad suite of PAHs was found in all samples (Table 3). With the exception of naphthalene, the prominent PAHs found in *ARROW* Bunker C were remarkably preserved

TABLE 3. Concentration of PAHs (ng/g) in shoreline samples from Black Duck Cove and Janvrin Lagoon as determined by GC/MS.

	Black Duck Cove					Janvrin Lagoon oiled beach sediment
PAH[a]	*ARROW* oil	Tar pavement interior	Fluid spit tar	Water over tar pavement	Intertidal lagoon water	
Naph	121 000	3 050	772	0.006	0.0007	BQL[b]
Fluor	55 000	4 560	4 370	0.003	BQL[c]	17.5
Phen	184 000	19 200	7 690	0.006	0.0015	BQL[b]
Anth	15 800	1 710	1 540	BQL[d]	BQL[c]	20.5
Flu-th	26 000	4 490	3 580	0.006	0.0017	43.5
Pyr	47 400	11 300	7 540	0.002	0.0027	5.0
BaA	38 300	4 370	2 940	0.004	0.0009	27.5
Chry	106 000	29 200	16 200	0.030	0.0053	22.5
BbjkF	25 400	7 110	3 360	0.009	BQL[c]	71.5
BaP	17 700	5 330	2 850	BQL[d]	0.0016	151.0
BeP	40 200	9 130	5 170	0.009	0.0016	10.0
DBahA	8 540	BQL[f]	BQL[f]	BQL[f]	BQL[c]	45.0
IP	5 690	BQL[f]	BQL[f]	BQL[d]	BQL[c]	11.0
BghiP	3 440	BQL[f]	BQL[f]	BQL[d]	BQL[c]	94.0
Total	705 000	99 400	56 000	0.075	0.0160	519.0

[a]Naph, naphthalene; Fluor, fluorene; Phen, phenanthrene; Anth, anthracene; Flu-th, fluoranthene; Pyr, pyrene, BaA, benzo[*a*]anthracene; Chry, chrysene; BbjkF, mixture of benzo[*b*]-, benzo[*j*]-, benzo[*k*]fluoranthene; BaP, benzo[*a*]pyrene; BeP, benzo[*e*]pyrene; DBahA, dibenzo[*a*,*h*]anthracene; IP, indeno(1,2,3-cd)pyrene; BghiP, benzo[*g*,*h*,*i*]perylene.

[b]BQL = below quantitation limit < 0.5 ng/g.

[c]BQL < 0.0005 ng/g.

[d]BQL < 0.001 ng/mL.

[e]BQL < 0.33 ng/g.

[f]BQL < 520 ng/g.

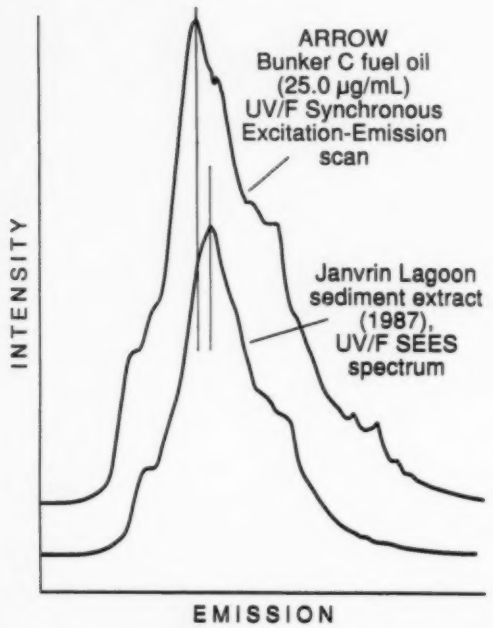

FIG. 4. Synchronous excitation-emission UV/F spectra of reference *ARROW* oil (1970) and oiled sediment from Janvrin Lagoon (1987), showing shift in emission peaks.

within the tar pavement and in an oil pool located within the Black Duck Cove cobble beach. Concentrations of PAH in the different environmental reservoirs (tar residue, sediment, water, tissue) differed by orders of magnitude: ppt concentrations for surface tars, low ppm for oiled sediments, and low ppb concentrations in water samples. Surprisingly, several low molecular weight and highly volatile compounds such as naphthalene (C10) were found in most samples.

Concentrations of residual hydrocarbons within coarse beach sediments from Janvrin Lagoon range from approximately 17 µg *ARROW* Bunker C equivalents/g sediment dry weight to as high as 4 mg/g. The F1 chromatogram shows a heavily eroded but nonetheless recognizable petrogenic *n*-alkane profile (Fig. 3A) overlying a large UCM. An earlier UV/F profile showed a typical spectrum, similar to original *ARROW* Bunker C, but with a slight shift to higher emission wavelength (Fig. 4).

Hydrocarbons in the beach sediments appear to be relatively strongly bound to sediments. Vigorous extraction into water of oiled sediments, as might occur by tidal action during a storm period, removed less than 0.1% of the total shown to be available by organic solvent extraction (Table 4).

Fingerprinting

All sediment samples contained the suite of triterpane stereoisomers characteristic of Bunker C fuel (Fig. 5). Also, comparison of normalized peak areas of pentacyclic triterpanes (Table 5) showed close correlation between oil residues from the study sites and the original Bunker C spilled from the *ARROW*. The combination of these criteria indicates that the oils have a common source. By comparison, they differed substantially in peak areas and mass fragmentograms from an unrelated oil (C & C Refinery oil). However, pentacyclic triterpane profiles from water extracts from both locales did not correspond to that of *ARROW* Bunker fuel.

TABLE 4. Hydrocarbon concentrations in (μg/g *ARROW* Bunker C equivalents) in sequential laboratory extractions of oiled beach sediment (Janvrin Lagoon) into freshwater, cold dichloromethane, and hot alkaline saponification (means of triplicate subsamples ± SD).

	Freshwater	Cold CH_2Cl_2	Hot KOH/CH_3OH
Total hydrocarbons	1.17 ± 0.26	1110.0 ± 174.0	16.7 ± 3.11

TABLE 5. Normalized areas (%) of pentacyclic triterpane peaks in petroleum residues determined by SIM (*m/e* = 191.3). All samples from Black Duck Cove except beach asphalt (Janvrin Lagoon), original *ARROW* Bunker C, and a C&C Refinery oil reference sample.

		Black Duck Cove					
	ARROW Bunker C	Subcrust tar	Fluid tar	Tar smear	Pavement washing	Janvrin Lagoon beach asphalt	C & C Refinery oil
A[a]	5.0	5.0	4.1	4.3	4.2	6.1	3.6
B[a]	4.6	4.5	3.9	4.2	4.0	4.8	5.6
C[a]	5.8	5.8	5.1	5.1	5.0	6.4	6.0
D 17α(H),21β(H)-30-Norhopane	17.0	18.0	17.0	18.0	16.0	17.0	17.0
E[a]	3.9	3.6	3.3	3.4	3.6	3.9	5.8
F 17α(H),21β(H)-Hopane	21.0	22.0	21.0	21.0	21.0	19.0	26.0
G 17β(H),21α(H)-Moretane	3.0	2.8	2.4	2.6	3.0	2.8	4.3
H 17α(H),21β(H)-Homohopane (22*S*)	11.0	10.0	11.0	11.0	11.0	10.0	8.3
I 17α(H),21β(H)-Homohopane (22*R*)	7.4	7.8	8.2	8.1	8.1	7.7	6.1
J 17β(H),21α(H)-Homomoretane (22*R*)	4.5	4.8	5.2	4.4	5.1	5.0	2.1
K 17α(H),21β(H)-Bishomohopane (22*S*)	6.2	5.6	6.5	6.3	6.2	6.0	5.4
L 17α(H),21β(H)-Bishomohopane (22*R*)	4.0	3.9	4.6	4.4	4.4	4.0	3.7
M 17α(H),21β(H)-Trishomohopane (22*S*)	4.5	3.7	4.7	4.5	4.6	4.3	4.0
N 17α(H),21β(H)-Trishomohopane (22*R*)	2.8	2.2	3.2	3.1	3.7	2.6	2.5

[a]Suite of triterpanes with characteristic GC distribution, unidentified.

Discussion

Fingerprinting Residues

One of the most vexing problems in studies of this kind is that of chemically fingerprinting the residues from spilled oils. Chemical alteration of oil residues of physical/chemical or biological weathering processes, the absence of appropriate chemical markers, the presence of coeluting biogenic hydrocarbons, or hydrocarbons of pyrogenic origin, and contamination by other anthropogenic or industrial hydrocarbon products can all confuse identity and origin of residual tars, especially of long-term weathered material. We ourselves encountered this problem in an earlier study of a Chedabucto Bay beach site, known to be oiled with *ARROW* Bunker C in 1970, but subsequently apparently contaminated with a product tentatively identified as No. 2 fuel oil (Keizer et al. 1978). In the present study, we focussed therefore on two known sites (Janvrin Lagoon, Black Duck Cove) where, besides the continuous visual record, we now have the evidence of pentacyclic triterpane profiles that indicate that the 1990 tar residues come from 1970 *ARROW* Bunker fuel. They have been used recently to distinguish *AMOCO CADIZ* hydrocarbon inputs from other sources (Page et al. 1988) and to trace North Sea oil slicks to their pipeline source (Grahl-Nielsen and Lygre 1990). These epimers are not known to occur as a result of biological synthesis but are found in petroleum and related sediments. Contamination from other hydrocarbon sources, anthropogenic or biological, is of course not ruled out, but we are at least confident that the bulk of the residual tar residues are remnant of the *ARROW* Bunker C fuel spilled there in 1970. Triterpane profiles for the water extracts from both locales were confused by the presence of other peaks (Fig. 5), most likely contributions from biological synthesis, or other sources. For example, peak O (eluting between 17β(H),21α(H)-homomoretane (22*R*) and 17α(H),21β(H)-bishomohopane (22*S*)) may well be diploptene (unidentified reviewer, personal communication) which is of biogenic origin, possibly bacterial. There were also slight variations in the relative contributions of the triterpanes.

Weathering and Persistence

The samples from the two study sites exhibit a surprising spectrum of degraded petroleum residues, from seemingly unaltered to severely weathered tars. Using the UCM boiling ranges given in Table 2, the various petroleum residues can be ranked in order of least to most weathered as follows: preserved *ARROW* Bunker C > visibly oiled beach sediment > fluid tar within cobble beach > interior of tar pavement > beach sediment (no visible oiling) > weathered tar smears, beach asphalt > exposed surface of tar pavement.

This confirms that, under low to medium energy conditions and for sediments of a certain grain size, spilled and stranded petroleum hydrocarbons can persist for a long time, under certain conditions in remarkably unaltered form.

Ours is not an isolated case, as in a similar study in Wild Harbor marsh (Massachusetts, USA), 20 yr after a No. 2 fuel oil spill from the barge *FLORIDA*, Teal et al. (1992) found trace amounts of No. 2 fuel hydrocarbons to a depth

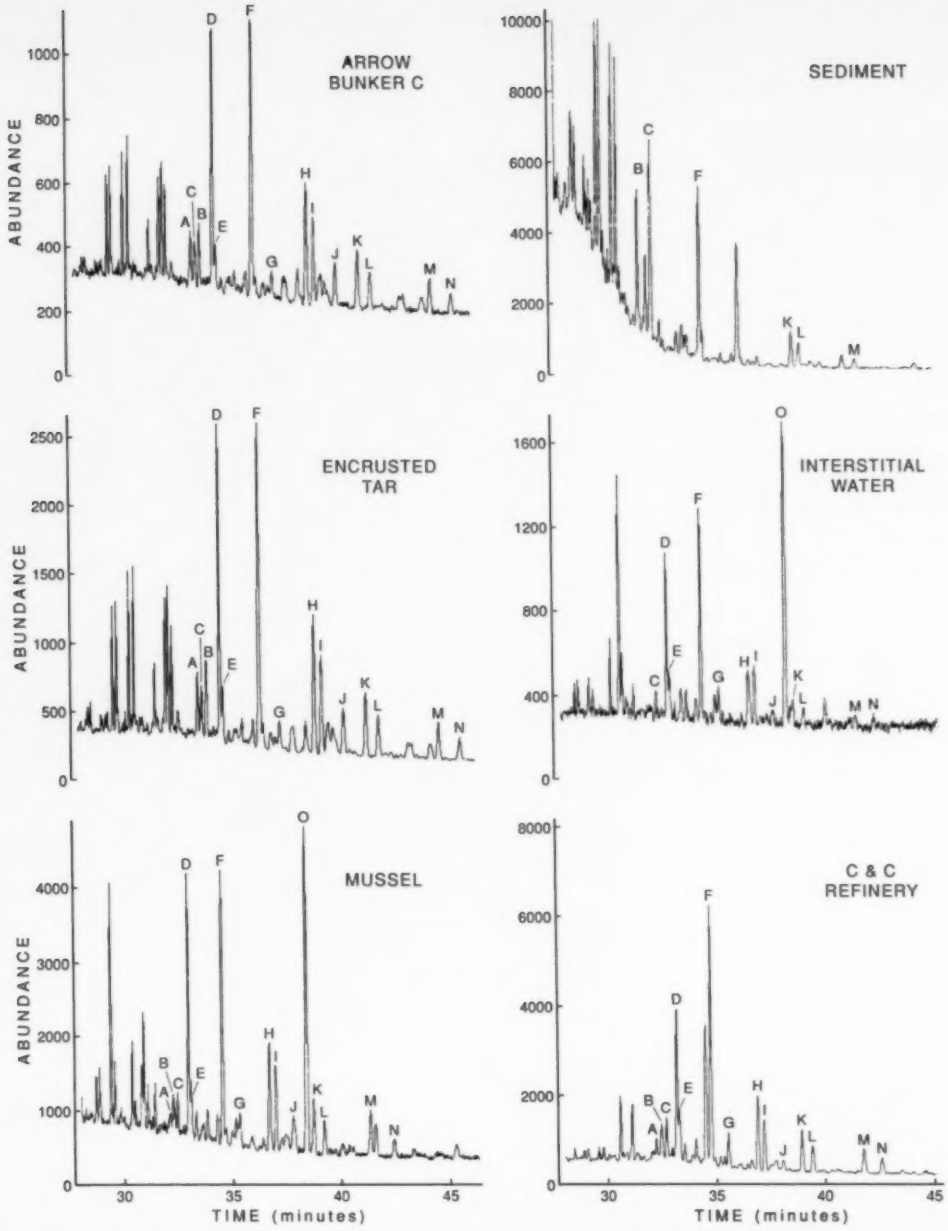

FIG. 5. Pentacyclic triterpane profiles for original *ARROW* Bunker C fuel oil, and 20-yr hydrocarbon residues in water, sediment, and mussels from Janvrin Lagoon. C & C Refinery oil is unrelated reference oil. A, B, C, E, O, unidentified triterpanels; D, 17α(H),21β(H)-30-norhopane; F, 17α(H),21β(H)-hopane; G, 17β(H),21α(H)-moretane; H, 17α(H),21β(H)-homohopane; I, 17α(H),21β(H)-homohopane; J, 17β(H),21α(H)-homomoretane; K, 17α(H),21β(H)-bishomohopane; L, 17α(H),21β(H)-bishomohopane; M, 17α(H),21β(H)-trishomohopane; N, 17α(H),21β(H)-trishomohopane.

of 15 cm. Hydrocarbons were also detected in several marsh faunal species, and fish (mummichog, *Fundulus heteroclitus*) taken from the marsh showed slightly elevated P4501A content. It remains uncertain whether this was really related to persisting No. 2 fuel type hydrocarbons. However, the Wild Harbor marsh hydrocarbon residues showed substantial weathering and biological degradation whereas in Chedabucto Bay, we found several tar residues that were relatively unaltered. The difference would seem to be that the relatively permeable nature of the Chedabucto Bay sediments, in contrast with the relatively impermeable nature of the Wild Harbor marsh sediments, permitted stranded oil to become sequestered and protected within shore sediments. Cobble beaches, such as the spit guarding Black Duck Cove, provide

extreme examples of this process, as they are very likely to entrap spilled oil. In fact, tar residues trapped within such permeable sediments are in low-energy microenvironments where they are likely to persist. Persistence then is a direct function of beach sediment permeability, of sediment grain size, of the depth to which entrapped tar residues penetrate. Persistence is an inverse function of the depth and frequency in which sediments are reworked during tidal incursions. Indeed, extrapment of oil within cobble beaches was feared to become a problem in Prince William Sound following the *EXXON VALDEZ* spill, but there the extreme reworking of the intertidal zone in the sound negated much of the potential for such entrapment and persistence.

The spectrum of gravimetric determinations in the various fractions of Chedabucto tar residues (Fig. 2) demonstrates the broad range in weathering of environmental oil residues, and potential persistence of a broad range of petrogenic PAH. Clearly, beach sediments on the one hand can and do act as a continuing long-term sink for such spilled hydrocarbons, especially of F2 aromatic components. On the other hand, interstitial hydrocarbons showed considerable loss of F1 aliphatics. This had also been described by Keizer et al. (1978) for other nearby beaches. This observation led to the speculation that medium- to fine-grained beaches act as "F1 filters" in the ecosystem.

The close resemblance to *ARROW* Bunker C of the fluid tar removed from the Black Duck Cove cobble spit and the subcrust tar from within the tar pavement indicates what is essentially unchanged Bunker fuel, at least as far as the relative weights of the various F1, F2, and uneluted fractions are concerned. The close match may have been influenced by the loss of some of the more volative hydrocarbons in the gravimetric technique (Farrington et al. 1976). However, the similarity, at least at this gross (gravimetric) level, remains high even after 20 yr weathering in a highly energetic environment. Similar persistence of low molecular weight hydrocarbon components after 13 yr was seen at the site of the 1974 *METULA* oilspill (Strait of Magellan) in residues collected in 1987 (Owens et al. 1988). In that case the residues come from above the spring high water line, and presumably were not subjected to repeated tidal washings as were the samples from the Chedabucto Bay locations. The slight shift in UV/F maximum with respect to original *ARROW* reference material was also seen by Teal et al. (1992) in their Wild Harbor marsh study; it is ascribed to a slightly increased content of three- to five-ringed PAH series found in weathered materials.

Of the different weathering processes, evaporation and dissolution obviously played major roles, as is evident in the differences between, for example, pavement crust tar and interior tar. It is more difficult to distinguish their relative importance in more heavily weathered residues such as beach asphalt and crust tar. Boehm et al. (1981) have suggested that weathering by evaporation can be distinguished from that by dissolution by a comparison of saturated and aromatic low-boiling compounds (because of the much larger solubilities of the aromatics). Because F1:F2 ratios have shifted over time, it is difficult to know whether that model applies here to these long-term persistent residues. Microbial degradation is indicated as a weathering agent in the fluid tar and the subcrust tar samples by reduced C17 to pristane and C18 to phytane ratios (data not shown here), as compared with that of the original oil (Atlas et al. 1981; Boehm et al. 1981). Residues in the heavily oiled sediments from Janvrin Lagoon may represent as case of extreme biodegradation, with even the isoprenoids broken down (Atlas et al. 1981). In this case also, there was significant biogenic input.

Sediment–Hydrocarbon Interaction

The resemblance of the 20-yr-old in-beach oil residues in Janvrin Lagoon to original *ARROW* oil rather than to weathered surface tar smears along the high-tide line, suggests a revision of an earlier interpretation of oiling of medium-grained (gravel–sand–silt) beaches (Vandermeulen and Gordon 1976). In the earlier model, an incoming oil slick was thought to be moved progressively higher up the beach slope, eventually remaining stranded along the upper high-tide line. Such surface-stranded Bunker C residues were thought to then become the long-term source for interstitual hydrocarbons by percolating downslope over the following years to contaminate the downslope and underlying sediments. We now think that today's interstitial petroleum residue derives mainly from oil slick material that first penetrated the beach face at the time of the spill. Over the subsequent years, this slick-derived material has remained entrapped within interstitial spaces since the original accident. No doubt, the surface-stranded oil/tar residue has contributed some of the in-beach hydrocarbons, but it probably represents a very small imput, and becomes progressively less so as it continuously weathers to tar. We therefore recognize two distinct environmental reservoirs of stranded oil residue in these medium- to coarse-grained beaches: the interstitial residue as the major source and surface-stranded residue as the minor source, each having its own weathering rate, history, and persistence. For example, the surface reservoir/residue is constantly exposed to oxidation, photolysis, and physical weathering (by wave action, sediment scouring, and tidal washing). The in-beach interstitial residue, however, is in large part protected from these processes (except for microbial degradation), except where interstitial sediments are deeply reworked during tidal incursions. One consequence of this reinterpretation is that this enables a different calculation of degradation rates and persistence of stranded oil (viz., e.g., Reed et al. 1989).

Ecological Consequences

This interpretation has considerable ecological significance, at least for similar spills in north-temperate environments. First, any postspill toxicological threat to intertidal fauna and flora probably depends primarily on the weathering state of interstitial hydrocarbons and not on the superficial surface-stranded residues. This shifts the focus for bioavailability of hydrocarbons away from surface-stranded oil to the interstitial residues as the real long-term threat. Second, estimates of persistence may well have to be revised upward to reflect the potentially higher persistence of interstitially sequestered petroleum residues. This is especially so in the case of such oiled beach systems as protected sand–gravel lagoons and deep cobble–boulder beaches, particularly those that undergo little sediment movement. Third, evaluations of coastal sensitivity for oilspill contingency planning should recognize the very long persistence of stranded oil residues that can be expected in coarse-grained and poorly sorted sediments and cobble beaches.

What then is the environmental threat to Chedabucto Bay

of these long-term persistent *ARROW* petroleum residues? As transferance of residual hydrocarbons, at this stage, from oiled beach sediment into tidal water is low (low ppb) (Table 3), even from tar pavements as in Black Duck Cove, the likelihood of contamination and toxicity arising from oil remobilization seems a relatively low risk. Also, where such remobilization does occur, much of the released material will be extensively weathered. The bioavailability and bioconcentration of these hydrocarbons will be dealt with in another study, but despite bioconcentration, the actual hydrocarbon levels in tissues (e.g., bivalves in Janvrin Lagoon) are low. As for such large environmental tar reservoirs as cobble-entrapped and protected fluid tar, again, the transfer of hydrocarbons into tidal waters is a very low level process, except for those rare and sporadic events when the entire cobble armor becomes reworked. In summary, then, from a hydrocarbon transfer point of view, these long-term deposists appear to act as relatively inactive sinks of hydrocarbons, being to a large part isolated from the aquatic ecosystem.

Conclusions

(1) Tar residues obtained from a 20-yr-old spill site exhibit a wide spectrum of degraded petroleum residues, from virtually unaltered to severely weathered tars. Pentacyclic triterpane profiles indicate their origin as the *ARROW* Bunker fuel spilled in 1970.

(2) These findings indicate that, under low to medium energy conditions and for relatively porous sediments, petroleum hydrocarbons can persist for decades. Residues bound in tar pavements and within cobble beaches probably persist for several decades. These periods substantially exceed the degree of persistence commonly assumed.

(3) Oil residues buried within beach sediments can retain even highly mobile low molecular weight components for up to 20 yr, and probably longer. This occurs despite their being repeatedly submerged by tidal invasion. However, remobilization of mobile hydrocarbons into the aquatic environment appears to occur very slowly.

(4) A model is proposed in which residual spilled hydrocarbons exist in two distinct environmental reservoirs: the interstitial residue as the major source and surface-stranded residue as the minor source, each reservoir having its own weathering rate, history, and persistence. In this scheme the remaining interstitial oiling of gravel–sand–silt beaches is derived mainly from oil slick material that penetrated the beach face immediately at the time of stranding or shortly after.

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Daily Settlement Rates of the Zebra Mussel, *Dreissena polymorpha*, on an Artificial Substrate Correlate with Veliger Abundance

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Martel, A., A.F. Mathieu, C.S. Findlay, S.J. Nepszy, and J.H. Leach. 1994. Daily settlement rates of the zebra mussel, *Dreissena polymorpha*, on an artificial substrate correlate with veliger abundance. Can. J. Fish. Aquat. Sci. 51: 856–861.

The relationship between daily settlement rates and local concentrations of veligers of the zebra mussel, *Dreissena polymorpha*, was investigated using plankton sampling and scouring pad collectors. A total of 102 plankton and 136 collector samples were taken from a nearshore site near Wheatley, Ontario, in west-central Lake Erie, over 17 consecutive d in August 1992. Results demonstrate a strong correlation between daily settlement rates and daily concentrations of late-stage (competent) veligers in the water column (determined at shell lengths ≥170 μm; $r = 0.93–0.98$; $p < 0.001$; $\log_{10}$-transformed data). Variations in settlement rates of 1–3 orders of magnitude occurred within 24–96 h. Results also suggest that wind-induced hydrodynamics can affect settlement rates of zebra mussel larvae; the period used to monitor settlement (24 h) was much shorter than that employed (~1–2+ wk) in previous studies. Daily monitoring of concentrations of late-stage veligers in the water column as well as settlers (fibrous collectors) may help to identify biological and physical factors affecting short-term variability in settlement; it may also prove advantageous for industries where early detection of colonization by the zebra mussel is critical for cost-effective control of this invasive mollusc.

La relation entre la concentration de larves véligères de la moule zébrée, *Dreissena polymorpha*, et leur taux de fixation quotidien a été étudiée à partir d'échantillons de plancton et d'individus fixés à des collecteurs fibreux (tampons à récurer) après une immersion de 24 h. L'étude a été conduite à Wheatley (Ontario), au lac Érié, pendant 17 jours en août 1992 (total de 102 échantillons de plancton et de 136 collecteurs). Les résultats démontrent une forte corrélation entre la concentration de véligères avancées (≥170 μm) et le taux de fixation quotidien ($r = 0,93–0,98$; $p < 0,001$; transformation logarithmique). Des variations de l'ordre de 10 à 1000 fois dans le taux de fixation ont été observées en l'espace de 24–96 h. Les résultats suggèrent aussi que les facteurs hydrodynamiques peuvent affecter les taux de fixation des véligères. L'intervalle de temps entre chaque échantillonnage (24 h) est plus court que celui utilisé dans les études antérieures (~1–2+ sem). La lecture quotidienne des concentrations de véligères avancées, de même que des taux de fixation sur collecteurs fibreux, peut s'avérer utile lors d'études sur les facteurs physiques et biologiques affectant les taux de fixation larvaire. Ce prélèvement quotidien d'échantillons peut aussi être utile aux industries, où la détection hâtive est critique dans le contrôle de ce mollusque.

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Larval settlement and the consequent influx of early juveniles play a major role in population recruitment and community structure of benthic invertebrates with planktonic larvae (Underwood and Denley 1984; Connell 1985; Gaines and Roughgarden 1985; Minchinton and Scheibling 1991). Larval settlement rates show large spatiotemporal variability (Caffey 1985), with daily, weekly, or biweekly variations of 2–3 orders of magnitude being reported in both marine invertebrates (Yoshioka 1982; Caffey 1985; Gaines et al. 1985; Bertness et al. 1992) and the zebra mussel, *Dreissena polymorpha* (Pallas) (Lewandowski 1982a; Bij de Vaate 1991; Fraleigh et al. 1993). One possible explanation is that spatiotemporal variability in settlement rates reflects spatiotemporal variability in larval supply, a hypothesis that is supported by empirical studies of both marine (Grosberg 1982; Yoshioka 1982; Gaines et al. 1985; Minchinton and Scheibling 1991; Bertness et al. 1992) and freshwater species (zebra mussel; Fraleigh et al. 1993; Garton and Haag 1993). A strong positive correlation between larval concentrations and settlement rates means that the former, which are easier to sample, can be used in building population models. Estimating settlement rates from estimates

of larval concentrations is particularly useful in cases (such as the zebra mussel) where the ability to predict larval settlement has important economic implications.

In most studies to date (including those on the zebra mussel), spatiotemporal variability in settlement has been investigated by monitoring densities of settlers on hard substrates (i.e., bedrock, plates, glass slides) at ~1- to 3-wk intervals (but see Bertness et al. 1992). Long intervals between sampling of settlement preclude determination of settlement patterns on shorter time scales. Moreover, physical factors such as winds and water currents, recognized as being potential sources of variability in settlement (see Yoshioka 1982; Wilson 1990; Gaines and Bertness 1992), are known to change both in speed and direction within a matter of hours. Thus, shorter sampling intervals for monitoring settlement (e.g., 24 h) may eventually provide useful information on the relation of physical factors to short-term variability in settlement. For fouling organisms such as the zebra mussel, daily monitoring permits rapid implementation of control measures that can be effective in the early stages of settlement, but are less effective once the population is well established (see Barton 1993).

We investigated the relationship between daily settlement rates and local concentrations of veligers of the zebra mussel at a nearshore site in west-central Lake Erie. Using plankton sampling and scouring pad collectors upon which late-stage veligers can settle in large numbers overnight (Martel 1992a, 1992b, 1993), we show a strong correlation between daily settlement rates and daily concentrations of veligers, especially late-stage veligers. This result emphasizes the need to consider larval supply in recruitment predictions or population models of the zebra mussel in the Great Lakes.

Materials and Methods

The study was conducted in the west-central basin of Lake Erie, near Wheatley, Ontario, at the Lake Erie Fisheries Station, Ontario Ministry of Natural Resources (42°03′N, 82°27′W). Samples of veligers (plankton) and settlers (collectors) were taken about 7–10 m away from the northwest side of the Wheatley breakwater (see Martel 1993, fig. 1) at a depth of 2–4 m (water depth off the exposed southeast side of the breakwater is ~6–7 m). We chose this site because it allowed daily sampling of both larval concentrations and settlement rates even during storms: waves were often 1.5–2+ m high on the exposed southeast side, compared with <1 m high on the northwest side.

Recent studies have shown that there is, in addition to *D. polymorpha*, one other species of dreissenid in the Great Lakes, called the "quagga" mussel (May and Marsden 1992; Pathy and Mackie 1993). However, adult shells collected in the vicinity of Wheatley match the description of *D. polymorpha* (unpublished data). The small size of individuals collected in this study (90–400 μm) precluded comparisons of shell shapes. Based on the adult data, we assume that collected specimens belong to *D. polymorpha*.

Larval Concentrations

Larval concentrations were determined by taking daily zooplankton samples using a bilge pump (model: Attwood, Bilge King 2000; maximum output: ~8140 L/h near surface or ~5680 L/h at 1.5 m deep). The 12-A bilge pump was operated from a 12-V marine battery (load test: 230 A), which allowed us to take the unit to the field site by boat and sample the water column near the location where scouring pad collectors were deployed. Personal observations indicate that the impeller did not cause physical damage to the prodissoconch (shell of the bivalve veliger). Similar conclusions have been reached by other investigators using similar pumps (see Marsden 1992).

Plankton samples of 30 L were taken near the Wheatley breakwater between collector stations 3 and 4 (Martel 1993, fig. 1) at a distance of ~10 m from each collector station. We calibrated the pump output by measuring the time (usually 22–24 s) required to fill the equivalent of a 30-L bucket. This time represented the duration of the sampling period at a predetermined depth of 1.5 m. Volume calibration was determined before each sampling series (twice a day). Six replicate plankton samples were taken every 24 h, three in the morning (9:30–10:30) and three in the afternoon (16:00–18:00), between August 11 and August 29, 1992 (N = 102 plankton samples). Each set of three samples was pumped consecutively at ~2- to 5-min intervals. Samples were filtered directly into a 12-L plastic bucket, which had had the bottom removed and replaced by a 64-μm-mesh Nitex™ net. The bucket and its contents were then carefully washed into 1-L glass jars. In the laboratory, larvae in the zooplankton samples were observed live under a stereomicroscope and then preserved in 70% ethanol for later measurements.

Daily Settlement of Veligers

Collectors consisted of white-coloured nylon kitchen scouring pads (12 × 11 cm and 0.6–0.8 cm thick; supplier: Fireco/Washrack Co., Mississauga, Ont.) (Martel 1992a, 1992b, 1993; also see King et al. 1990 for the use of scouring pad collectors in monitoring *Mytilus* settlement). When late-stage veligers are present in the plankton, deployment periods as short as 24 h are sufficient to allow larvae (including early postmetamorphic stages during windy periods; Martel 1993) to settle in large numbers (often 100–1000+ settlers·pad^{-1}·d^{-1}). Moreover, when deployed for short periods (e.g., 24 h), scouring pads remain clean and collect only late-stage, competent veligers with shell length ≥165–170 μm (umbo-ventral margin measurements) (Fig. 1B). Veligers <165–170 μm, although often numerically dominant in the plankton (Fig. 1A), pass through the fibres of the collector without attaching or settling (Fig. 1B). At this early stage, veligers are not ready or competent to settle and metamorphose. In contrast, the settlement and metamorphosis of late-stage veligers (established at ≥170 μm) was confirmed by examination of collectors under a high-power stereomiscroscope. Numerous newly settled individuals were found to be attached to the nylon fibres of the collectors by their microscopic attachment byssus; furthermore, 90–100% of these settled larvae had metamorphosed, i.e., had lost their velum. This indicated that, following contact with the pad's nylon fibres, the veligers had settled, secreted byssi, and had time to metamorphose within the 24-h deployment period.

To determine daily settlement rates, four replicate collectors were attached to an anchored polypropylene line at 40-cm intervals, with the first positioned at about 50 cm above the bottom. The line was kept taut by a small buoy just below the surface, which was attached to a larger, surface buoy. Two such lines were installed at two sites on the west

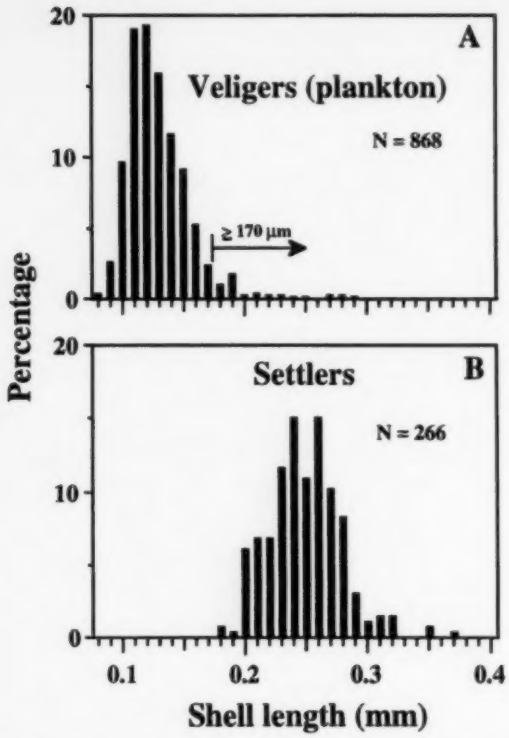

FIG. 1. (A) Size–frequency distribution of zebra mussel veligers (shell lengths; umbo-ventral margin measurements) from zooplankton samples taken on the west side of the Wheatley breakwater, lake Erie, at a depth of 1.5 m (N = 6 plankton samples of 30 L each: three samples taken in the afternoon of August 26 and three in the morning of August 27, 1992). Notice the relatively low percentage of mature, late-stage veligers (≥0.170 mm or 170 µm, indicated by the arrow) at the time of sampling. (B) Size–frequency distribution of newly settled individuals on scouring pad collectors after a 24-h deployment (from noon on August 26 to noon on August 27, 1992) (N = 8 collectors).

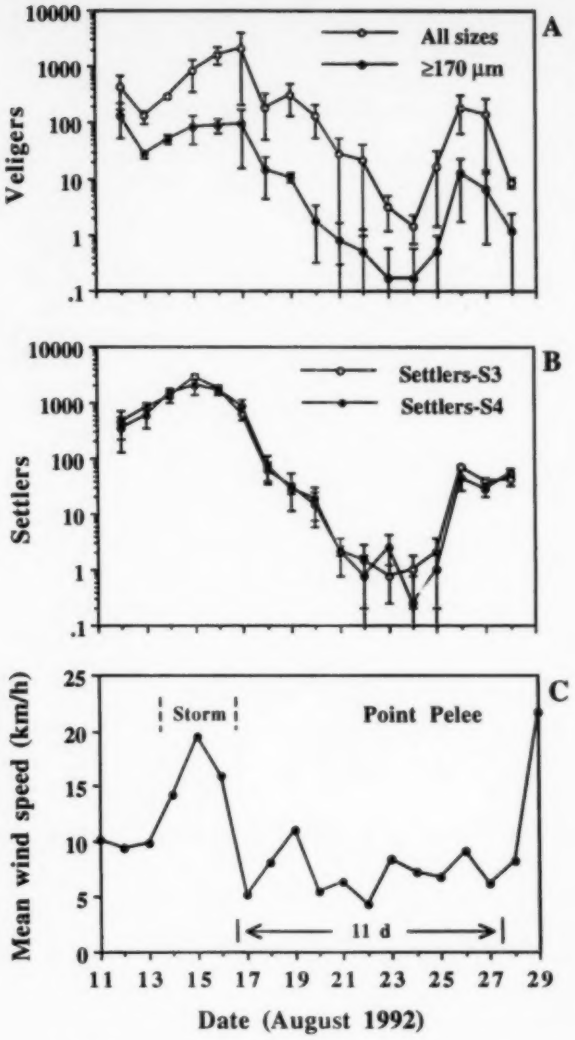

FIG. 2. (A) Mean daily concentrations of zebra mussel larvae per 30-L sample taken on the west side of the Wheatley breakwater during 17 consecutive d during August 1992. Each point represents the mean of six plankton samples: three taken in the afternoon and three taken in the morning of the following day (open circles: veligers of all sizes; solid circles: veligers ≥170 µm shell length). (B) Mean daily settlement rates of veligers on scouring pad collectors at two stations (deployment: from noon to noon the following day) (N = 4 collectors per data point). Open circles: station 3; solid circles: station 4. Vertical bars represent ±1 standard deviation. (C) Mean daily wind speed (km/h) recorded by the Point Pelee weather station during the study. The two arrows indicate the poststorm 11-d period of fair weather.

site of the Wheatley breakwater (collector stations 3 and 4; Martel 1993, fig. 1). Collectors were deployed daily at noon, between August 11 and August 29, 1992, with each deployment lasting 24 h (total of 136 collector samples). Extraction and filtration procedures for settlers followed the method described by Martel (1992a). Both settlers and planktonic veligers were preserved in 70% ethanol and later counted and measured (umbo-ventral margin measurements) using an Olympus SZH stereomicroscope (64×) equipped with a drawing tube and projected on a Jandel/SigmaScan digitizer system.

Wind data were obtained from the Point Pelee weather station (Environment Canada). Daily estimates of wind speed were obtained by calculating the mean of hourly wind velocities (kilometres per hour) prevailing during the time collectors were deployed.

Results

Daily settlement on scouring pad collectors reflected the concentrations of veligers at both collector stations (Fig. 2A and 2B). There were two main settlement episodes, with the first occurring at the beginning of the study (August 12–17; mean: 402–2518 settlers·coll.$^{-1}$·d^{-1}; Fig. 2B). Four days later, however, settlement rates had dropped by 3 orders of magnitude (August 21–25; mean: 0.25–2.50 settlers·coll.$^{-1}$·d^{-1}). At the end of this low-settlement period, settlement increased by approximately 30-fold within 24 h (August 26–28;

mean 28.7–69.7 settlers·coll.$^{-1}$·d^{-1}). Also, during the 17-d period, the mean concentration at late-stage veligers (≥170 μm) ranged from 0.17 to 137.5 ind./30 L (or from 5.7 to 4583 ind./m^3), while that of all stages of veligers ranged from 1.5 to 2064 ind./30 L (or from 50 to 68 800 ind./m^3) (Fig. 2A).

Linear regressions on $\log_{10}$-transformed data (see Yoshioka 1982) showed that at both stations, 87–91% of the variability of settlement rates was explained by variation in larval concentrations of late-stage (competent) veligers (r = 0.93–0.95; $p < 0.001$; Fig. 3). Moreover, regression models between the two collector stations were strikingly similar, with no significant difference between elevations (among adjusted means) or between slopes (ANCOVA; differences among adjusted means: $F = 0.168$; $p > 0.05$; differences among slopes; $F = 0.020$; $p > 0.05$; see regression models on Fig. 3). Larval concentrations and settlement rates were also positively correlated when all stages of veligers were used in the analysis, although in that case, a smaller amount of the variability in settlement rates (71–72%) was explained by variation in larval concentrations (r = 0.84–0.85; $p < 0.001$)

The highest settlement rates occurring at the beginning of the study (Fig. 2B) (1000+ settlers/pad) coincided with the arrival of a major low-pressure weather system, with high, long-fetch northeast winds (Fig. 2C). During this storm (August 13–16), the long fetch of the northeasterly winds caused increased wave action (1.5–2 m; unpublished data). When only the 11 d that followed the end of the storm are used (fair weather, mostly calm and sunny, with low winds) (Fig. 2C), correlations between settlement rates and concentrations of the late-stage veligers were improved at both stations (r = 0.95–0.98; Fig. 3). In the regression model from station 3, 97.5% of the variability of settlement rates is explained by the variability in larval concentrations of late-stage veligers ($p < 0.001$; Fig. 3). Regression models obtained from the two stations of the 11-d period also showed no significant difference between adjusted means or between slopes (ANCOVA; differences among adjusted means: F = 0.372; $p > 0.05$; differences among slopes: $F = 0.241$; $p >$ 0.05; Fig. 3).

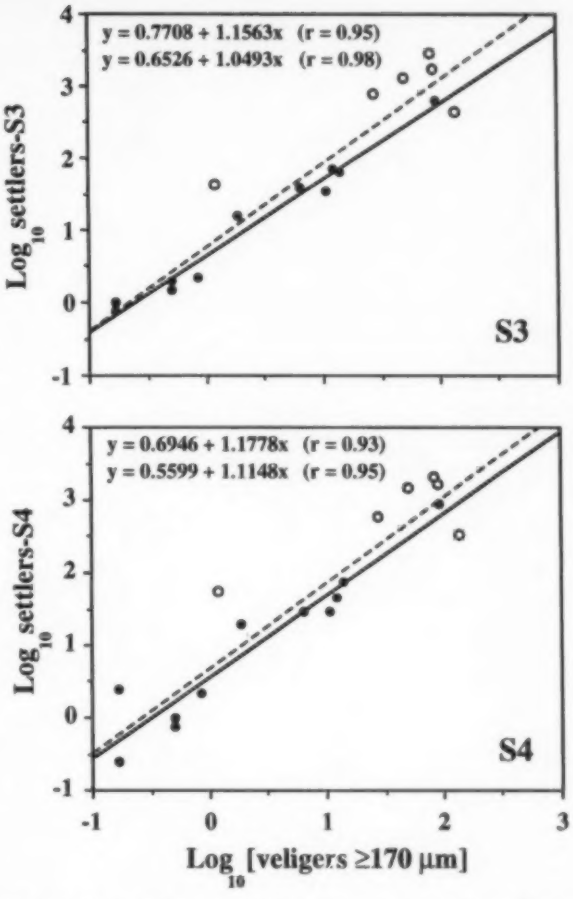

FIG. 3. Linear regressions ($\log_{10}$–$\log_{10}$) of mean daily settlement rates of zebra mussels at stations 3 (top panel) and 4 (bottom panel) as a function of mean concentrations of late-stage veligers (shell length ≥170 μm). The regression equation and the coefficient of correlation (r) are indicated at the top left corner. Open circles: pre- and during storm settlement; solid circles: settlement for an 11-d poststorm period. Broken line: regression based on all data; solid line: regression based on the 11-d poststorm period only.

Discussion

Daily settlement rates of the zebra mussel were highly correlated with local concentrations of planktonic veligers. These findings are consistent with previous studies on benthic invertebrates with planktonic larvae where settlement rates and larval abundance were monitored on a weekly, biweekly, or monthly basis (marine invertebrates: e.g., Yoshioka 1982; Gaines et al. 1985; Bosselmann 1989; Minchinton and Scheibling 1991; also see study by Bertness et al. 1992 on daily settlement of barnacles; freshwater (zebra mussel): Fraleigh et al. 1993; Garton and Haag 1993). Although there was a strong correlation (r = 0.84–0.85) between settlement and concentrations of all stages of veligers, it improved substantially (r = 0.93–0.95) when only late-stage veligers were included in the analysis. This improvement was expected, since only competent, developed veligers can settle on substrates and metamorphose (Lewandowski 1982b; Sprung 1989); the same is true of many marine benthic invertebrates (barnacles, using only cyprids: e.g., Grosberg 1982; Gaines et al. 1985; Bertness et al. 1992; bryozoans, only cyphonautes >350 μm: Yoshioka 1982). While daily concentrations of all stages of veligers and those of late-stage veligers followed similar trends (Fig. 2A), this situation can vary depending on size distribution of veligers in the plankton, which, in turn, can show wide spatial and temporal variations (e.g., early versus late in the breeding season) (see Lewandowski 1982a). Previous studies on the zebra mussel comparing settling rates with veliger abundance (e.g., Fraleigh et al. 1993; Garton and Haag 1993) did not discriminate between early- (noncompetent) and late-stage (competent) veligers. Measuring concentrations of the latter gives more accurate estimates of the number of veligers potentially capable of settling out at any given time. This is especially important when relating settlement rates to larval supply.

As pointed out by Keough and Downes (1982), the period immediately following settlement and metamorphosis may involve heavy mortality and should be considered when studying factors affecting distributions of adults, or when

building population models (Connell 1985). Because we submerged souring pad collectors for only 24 h, there is unlikely to be much mortality during this brief sampling interval (see Connell 1985). Consequently, estimates of settlement rates are probably not confounded by postsettlement mortality, as may be the case when collectors are deployed for long periods (e.g., many days, weeks, months).

Variability in settlement may arise from factors affecting abundance or larvae in the water column, such as the timing of breeding of adults (marine: Cameron and Rumrill 1982; King et al. 1989; Rumrill 1989; Martel and Chia 1991; freshwater (zebra mussel): Lewandowski 1982a; Garton and Haag 1993) and hydrodynamics (marine: e.g., Bousfield 1955; Yoshioka 1982; Shanks and Wright 1987; Gaines and Bertness 1992) (see also Lewandowski 1982a for effects of winds on spatial distribution of zebra mussel veligers). In this study, settlement rates of late-stage veligers at both stations varied by 1–3 orders of magnitude within 24–96 h, but the causes of this observed short-term variability are unknown. It is possible that nearshore wind-driven surface currents transport veligers and contribute to heterogeneity in distribution (patchiness), causing rapid fluctuations in local concentrations of larvae (see Legendre and Demers 1984 for a discussion of factors affecting spatiotemporal variability in zooplankton distribution).

There are few quantitative studies on the effects of hydrodynamics on settlement rates of planktonic larvae. Eyster and Pechenik (1987) reported a two- to eightfold increase in the percentage of attachment of *Mytilus edulis* veligers on filamentous substrates (algae and byssus of adults) during periods of water agitation. They suggested that water movement might increase the frequency of contacts between larvae and the filaments of the substrate. In this study, post-storm data (coinciding with the arrival of a high-pressure system and a period of calm weather) yielded the strongest correlation between settlement rates and concentrations of late-stage veligers. During the storm, resuspension of early postmetamorphic stages and their settlement on collectors (Martel 1993) also contributed to high settlement rates (unpublished data). Further studies involving various water movement conditions, as well as substrates of different composition and structural complexity (e.g., plates versus fibrous collectors), will be needed before the effects of hydrodynamics on settlement rates of zebra mussel larvae are clearly understood.

In this study, no data were obtained from natural (e.g., shells and byssus of adults, rocks, shells of unionids; see Hebert et al. 1991 and Mackie 1991) or man-made substrates (e.g., dams, water channels, pipes), although general patterns of settlement on scouring pads may reflect those on natural or man-made substrates. King et al. (1990) used similar flat nylon scouring pad collectors to study settlement of *M. edulis* on the west coast of Ireland and concluded that the use of such artificial substrates was useful in monitoring the mussel recruitment cycle insofar as settlement events on the pads mirrored those on filamentous algae and adult beds at the same site (see King et al. 1990). A similar study should be conducted on *D. polymorpha* in the Great Lakes to test how patterns of settlement on fibrous collectors as well as traditional plates compare with those on natural substrates.

Nonetheless, settlement rate variations of 1–3 orders of magnitude occurring within 24–96 h, as we found, suggest that daily monitoring of larval concentrations and settlement rates on scouring pad collectors or similar fibrous substrates may be of considerable benefit in cases where early detection of settlement is crucial to effective management. Because it is easier to deter zebra mussel veligers from settling inside water pipes than it is to rid pipes of attached juveniles or adults (Barton 1993), daily sampling of larval concentrations and settlement on fibrous collectors could allow for more efficient, cost-effective preventive measures.

Finally, the demonstration of a strong correlation between settlement rates on scouring pad collectors and abundance of late-stage veligers implies that larval supply plays a crucial role in the structure of zebra mussel populations, as has been demonstrated in some marine benthic invertebrates (Grosberg 1982; Yoshioka 1982; Minchinton and Scheibling 1991). The supply of veligers, especially late stages, should therefore be considered an important factor when trying to predict recruitment or colonization rates, or when developing population models of the zebra mussel in the Great Lakes.

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Interannual Variability of Nitrogen Limitation in a Desert Lake: Influence of Regional Climate

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A systematic evaluation of limnological and experimental indicators of nutrient limitation in Pyramid Lake during 1989–92 clearly show that N is the nutrient most limiting to phytoplankton. This conclusion is supported by dissolved inorganic nutrient ratios, seston elemental composition, nutrient enrichment bioassay experiments, and blooms of N_2-fixing algae. However, the degree of N limitation expressed during the three years varied considerably. A comparison of interannual patterns of N limitation with weather data collected at the lake suggests that differences among years can be attributed to climatic variations. Our study indicates that climatic variations affect N availability by altering the timing of winter deep mixing, varying the potential for upwelling during spring, and varying the rate of spring warming of surface waters. Summer wind patterns also appear to be an integral factor controlling initiation, development, and collapse of the annual bloom of *Nodularia spumigena*, a N_2-fixing blue-green alga. The results of this study emphasize the need to consider climatic variations in assessing the general nutrient status of lakes.

Une évaluation systématique d'indicateurs expérimentaux et limnologiques de la limitation des éléments nutritifs dans le lac Pyramid entre 1989 et 1992, a clairement montré que le N est l'élément nutritif qui limite le plus la prolifération du phytoplancton. Cette conclusion est confirmée par les mesures et rapports entre les éléments nutritifs inorganiques dissous, la composition élémentaire du seston, les essais biologiques d'enrichissement en matières nutritives et l'examen des proliférations d'algues fixatrices du N_2. Toutefois, le degré de limitation par le N a considérablement varié au cours des trois ans qu'a duré l'expérience. Le rapprochement des fluctuations interannuelles de la limitation du N avec les données météorologiques obtenues dans le secteur du lac, montre que les différences d'une année à l'autre sont attribuables à des variations climatiques. Notre étude indique que ces dernières modulent la disponibilité du N par le déplacement de la période de mélange hivernal des eaux en profondeur, par leur action sur le potentiel de remontée de l'eau au printemps ainsi que par la variation de la vitesse de réchauffement des eaux superficielles au printemps. La configuration estivale des vents paraît être aussi un facteur intégral du contrôle de l'amorce, du développement et de l'effondrement de la prolifération annuelle de *Nodularia spumigena*, une algue bleue fixatrice du N_2. Les résultats de cette étude mettent en évidence la nécessité de tenir compte des variations climatiques dans l'évaluation du bilan nutritif général des lacs.

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One of the cornerstones of limnological research is the relationship between nutrient availability and algal growth (e.g., Goldman 1960; Edmondson 1972; Vollenweider 1976; Schindler 1988). Generally, phytoplankton production in lakes is considered to be limited by the availability of P (Schindler 1977). However, in semiarid and mountainous regions in the western United States, N may be of equal or greater importance in limiting phytoplankton growth (Goldman and Horne 1983; Morris and Lewis 1988; Thornton and Rast 1989; J.E. Reuter et al., unpublished data). Elser et al. (1990) recently reviewed the existing bioassay literature on P and N in lakes of North America and concluded that, in freshwaters, N as a limiting factor may have a more important role than previously recognized.

A variety of indicators has been developed to infer nutrient limitation in aquatic systems including nutrient concentration, physiological status of plankton, enhanced growth in experimental assays and mesocosm enclosures, and whole-lake manipulations (Schindler 1975, 1977; Healey and Hendzel 1980; Vincent 1981a, 1981b; Lean et al. 1989; Dodds and Priscu 1990; Downing and McCauley 1992). Clearly, the optimal evaluation of limitation would be done using a combination of indicators including experiments in large enclosures (Hecky and Kilham 1988). Large-scale experimentation, however, is generally not logistically feasible in large lakes due to strong physical forcing, and determinations of limitation are usually based on indicators such as nutrient concentrations, physiological status, and growth bioassays. Given the difficulty in conducting large-scale experiments in large lakes, Dodds and Priscu (1990) suggested that the strongest conclusions about limitation can be made using growth-based parameters such as carbon production, chlorophyll, and particulate N and P.

Nutrient limitation of phytoplankton production in lakes has received a great deal of attention, which has resulted in an improved understanding of the link between nutrients and eutrophication. This, in turn, has facilitated improved management of aquatic resources. One area that has received less attention is how climatic variability affects nutrient

availability. Long-term monitoring of nutrients and phytoplankton production at Lake Tahoe and Castle Lake in the western United States has shown that interannual variations in annual production can be attributed to meteorological forcing (Goldman et al. 1989). Further, the depth of winter deep mixing in Lake Tahoe, which is controlled by winter weather conditions, was found to be a major source of both interannual variability in primary production (Goldman and Jassby 1990) and the stimulatory response of phytoplankton in summer bioassay experiments (Goldman et al. 1993). These studies, as well as others (e.g., Wetzel 1981), emphasize the importance of variations in meteorological forcing in lake ecology.

In this paper, we report the results of nutrient limitation studies performed in Pyramid Lake between October 1989 and December 1992. We have utilized several indicators of potential nutrient limitation including (1) water column ratios of dissolved and total N and P, (2) nutrient enrichment bioassays using natural phytoplankton populations, (3) seston ratios of C, N, and P, and (4) the appearance of N_2-fixing organisms in the phytoplankton assemblage. Reuter et al. (1993) provided a summary for the first year of this study and we build upon that initial framework to examine interannual variations in the degree of N limitation and how they may be related to climate.

Study Site

Pyramid Lake is a large (450 km^2), terminal, saline lake in western Nevada, U.S.A., with a total dissolved solids concentration of approximately 5300 $mg \cdot L^{-1}$. Lebo et al. (1992, 1993) described the study site in detail. Therefore, we restrict our description here to aspects of the region that are pertinent to a discussion of regional climate and nutrient limitation. The lake is 40 km long and its width varies from 6.5 to 16 km. Pyramid Lake is a deep, graben lake in the rain shadow of the Sierra Nevada mountains in eastern California and western Nevada, which greatly reduces atmospheric deposition of nutrients to the lake surface. At present, maximum and average depths are 102 and 60 m, respectively.

Pyramid Lake is a warm, monomictic lake, although temporary meromixis can occur during periods of high Truckee River inflow (Galat and Verdin 1988). The lake mixes during winter (December–February) and is thermally stratified at 20–25 m between June and October (Galat et al. 1981; Lebo et al. 1992). As a desert lake, Pyramid is P rich and N deficient (Hutchinson 1937; Galat et al. 1981) with a dissolved inorganic elemental N:P ratio generally <2 (Reuter et al. 1993). The shores of the lake are sparsely vegetated and mountainous, and the lake is subject to intense dust storms primarily during spring (Galat et al. 1981). When compared with other saline lakes, Pyramid Lake is oligotrophic with chlorophyll concentrations typically <3 $\mu g \cdot L^{-1}$ (Galat et al. 1981; Lebo et al. 1992). The phytoplankton assemblage is generally dominated by diatoms during the early spring, diatoms and green algae during summer, and blooms of *Nodularia spumigena* during summer–fall in certain years (Galat and Verdin 1988, 1989; Galat et al. 1990).

Methods

Water samples were collected monthly between October 1989 and December 1992 from the center of Pyramid Lake. Sampling includes Hydrolab profiles for physicochemical variables and collection of discrete samples for chemical and biological constituents. In this paper, we will focus on the aspects of the sampling program that relate to the determination of the nutrient status of the lake. Discrete water samples were collected from 11 depths throughout the 102-m water column for analysis of nutrient and particulate matter concentrations and from 10 depths in surface waters (0–25 m) for chlorophyll. Samples for nutrient analyses were stored frozen until analysis within a few months.

Nutrient concentrations were determined spectrophotometrically using standard colorimetric methods. N fractions were determined as follows: ammonium (NH_4-N) by modified indophenol reaction (Solorzano 1969; Liddicoat et al. 1975), limit of detection (LOD) of 2 $\mu g\ N \cdot L^{-1}$; nitrate + nitrite (NO_3-N) by cadmium reduction using a shaking technique (Jones 1984), LOD of 1 $\mu g\ N \cdot L^{-1}$; dissolved inorganic N (DIN) as the sum of NH_4-N and NO_3-N; total Kjeldahl N (TKN) as NH_4-N after acid digestion of raw sample (APHA 1985), LOD of 15 $\mu g\ N \cdot L^{-1}$; and total N (TN) as the sum of TKN and NO_3-N. P fractions were measured as soluble reactive P (SRP, Strickland and Parsons 1972). Total P (TP) was measured in a raw water sample following acid-persulfate digestion (APHA 1985), while dissolved reactive P (DRP) was measured in the 0.45-μm filtrate. TP and DRP analyses were modified to remove arsenic interferences (Johnson 1971). LOD for P analyses was 1–2 $\mu g\ P \cdot L^{-1}$. Dissolved silicate was measured as reactive silicate (APHA 1985) in the 0.45-μm filtrate (LOD of 50 $\mu g\ Si \cdot L^{-1}$).

Particulate matter was collected on Whatman GF/F filters (nominal 0.7 μm) for chlorophyll (Chl *a*) and seston C (PC), N (PN), and P (PP). Filters for PC/PN and PP were prebaked at 450°C for 2 h to reduce organic contamination. Chl *a* was measured fluorometrically after homogenization and a 24-h extraction into cold (4°C) 90% acetone (APHA 1985). Chl *a* concentrations were corrected for phaeopigments. PC and PN were determined using a Perkin Elmer model 2400 CHN analyzer (LOD of 2 μg N and 10 μg C). PP was determined as SRP after high temperature combustion (450–500°C) for 2 h followed by hydrolysis with dilute HCl (Solorzano and Sharp 1980, LOD of 0.02 μg P).

Nutrient enrichment experiments were conducted to assess the response of Pyramid Lake phytoplankton to additions of inorganic N, P, and Fe. Because our bioassays used the phytoplankton communities present in Pyramid Lake rather than a single test species, the results should be directly applicable in evaluating nutrient controls of natural populations in Pyramid Lake (e.g., Goldman 1978; Schelske 1984). Nutrient additions included DIN (NH_4-N or NO_3-N), DRP, Fe (Fe-EDTA), and DIN added with either DRP or Fe-EDTA. For Fe treatments, EDTA was used as a complexing agent so that Fe^{3+} would remain in solution and available to phytoplankton.

Bioassay experiments were conducted on 19 occasions during the 1989–92 study period. From February to October 1990, experiments were conducted monthly and then on six and three occasions during 1991 and 1992, respectively. A single experiment was conducted in 1989 at the time of the November sampling. For each assay, sample water was collected in 4-L cubitainers from a depth of 5 m in the center of the lake in conjunction with monthly samplings and transported to our laboratory in Davis, California, within 12 h. Lake water was maintained at the approximate temperature

TABLE 1. Mean, SD, and range of nutrient and particulate matter concentrations ($\mu g \cdot L^{-1}$) in Pyramid Lake for October 1989 – December 1992. Values are based on volume-weighted averages for monthly sampling of surface waters (0–20 m) in the center of the lake. The value for SD listed is for average monthly values for concentrations and ratios and does not propagate error from individual sampling dates. All ratios are in weight units. See text for additional information and abbreviations.

Nutrient fraction	Mean	SD	Maximum	Minimum	Sample number
PC	420	167	1040	131	37
TN	685	59	881	541	27
DON	626	59	802	445	27
DIN	17.9	13.9	60	3	39
PN	39.2	12.4	76	17.4	37
TP	81.5	7.7	97	62	33
DRP	63.5	8.0	78	46	35
PP	5.2	1.6	10.7	2.7	38
TN:TP	8.7	1.2	10.8	6.4	21
DIN:DRP	0.28	0.20	0.89	0.05	35
PC:PN	11.3	4.3	25.3	6.0	37
PN:PP	7.9	2.1	13.8	4.2	37

of collection and stored in the dark during transportation. The average difference between Chl *a* samples filtered at the time of field sampling and initial values for bioassay experiments was 36%, indicating that handling and processing probably affected algal composition. However, the purpose of the experiments was to assess the general nutrient status of phytoplankton. The results of the experiments fulfill that purpose, despite the observed changes in Chl *a* during handling.

Experiments were run at ambient lake temperature with a 12-h light period per day. Treatment flasks were filled with 450 mL of lake water prescreened (80 μm) to remove zooplankton grazers. The only exception was September 1990 when *N. spumigena* comprised a large fraction of the phytoplankton assemblage. For that date, whole lake water was used. Nutrients were added to five sets of flasks leaving one set as controls. Samples were taken for Chl *a* and nutrients analyses at the beginning and end of 5- to 6-d incubations. Chl *a* concentrations in treatments and controls at the end of the experiment were compared via Student's *t*-test to evaluate the algal growth response to nutrient additions.

Potential nutrient limitation of phytoplankton in Pyramid Lake was determined using both limnological and bioassay data. Limnological data used to assess potential limitation included the ratio of TN to TP, the ratio of DIN to DRP, and the elemental composition of seston (C, N, and P). For each monthly sampling date, volume-weighted average values for each constituent and ratio of constituents were calculated for euphotic waters (0–20 m) using concentrations for discrete samples collected at 0, 5, 10, and 20 m. These average values were used to assess potential limitation and calculate mean values. All ratios were calculated using concentrations expressed as weight per unit volume.

Meteorological data collected by Pyramid Lake Fisheries at Sutcliffe, Nevada, were used to examine climatic differences among 1990, 1991, and 1992. To assess the general trends of each year, average monthly values were calculated for all of the weather station variables reported. To reduce the total number of variables, we derived a single midpoint temperature variable. Midpoint temperature is defined as the midpoint between daily maximum and minimum readings with the daily range typically ±8°C relative to the midpoint. Weather variables used include midpoint temperature, precipitation, morning wind speed, and number of cloudless days. Because morning wind speed is often much lower than winds observed in the afternoon, we restrict our evaluation of wind data to general trends. Additionally, we ignore wind direction as a variable because prevailing winds are out of the north and west, which is a favorable direction for upwelling in the north end of the lake.

Results

Limitation Indicators

Potential nutrient limitation in aquatic systems is often evaluated by comparing the TN:TP ratio with a typical value for nutrient replete phytoplankton. One commonly used value for phytoplankton biomass is the Redfield ratio of 7.2 for N:P (Redfield et al. 1963). The TN:TP value in Pyramid Lake, however, is a poor predictor of overall nutrient availability for phytoplankton (Reuter et al. 1993). During 1989–92, mean TN:TP in surface waters (0–20 m) was 8.7, with values ranging above and below the Redfield ratio (6.4–10.8, Table 1). These values indicate that phytoplankton growth in Pyramid Lake should be balanced with respect to N and P. This low potential for N limitation indicated by TN:TP contradicts both previous work at Pyramid Lake and other indicators examined here and is due to high concentrations of dissolved organic N (DON), which averaged 626 $\mu g \cdot L^{-1}$ during the study period (82–98% of TN). Although the bioavailability of DON in Pyramid Lake is unknown, it is probable that a majority of DON (e.g., Harrison 1978), and hence TN, is unavailable to phytoplankton. It is noteworthy that little variation in DON concentrations was observed during 1989–92 when new sources such as river inflow and phytoplankton production were low. This supports the hypothesis that a large fraction of DON is unavailable. The opposite is true for TP where DRP comprised a majority (78%) of the total pool.

The ratio of dissolved inorganic nutrients indicated a strong potential for N limitation of phytoplankton at all times of year. The mean value of the DIN:DRP ratio was

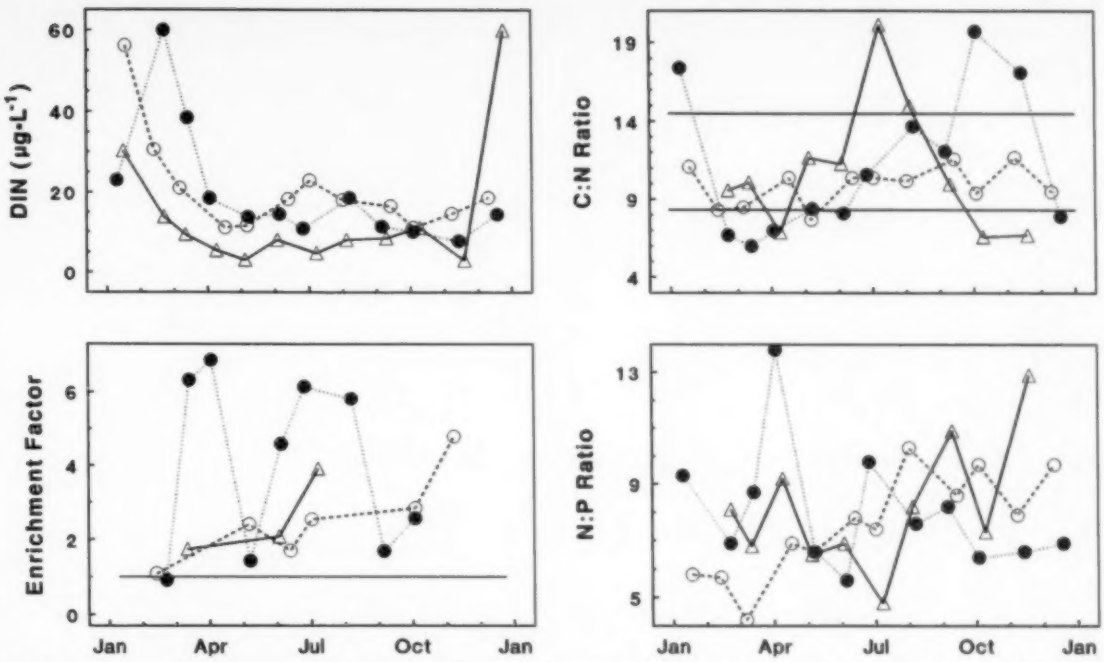

FIG. 1. DIN, bioassay enrichment factor for +50 µg N·L^{-1} treatment, C:N ratio of seston, and N:P ratio of seston for 1990 (●), 1991 (○), and 1992 (△). Data are plotted versus time of year. The horizontal line in the lower left panel indicates no stimulation. The horizontal lines in the upper right panel indicate regions of severe (>14.6), moderate (8.3–14.6), and no (<8.3) N deficiency (Healey and Hendzel 1980).

only 0.28 for the study period with a maximum value of 0.89 during February 1990 (Table 1). Throughout most of the year, DIN:DRP was generally <0.4 and only increased to values >0.6 during winter mixing. These seasonal variations in DIN:DRP were associated with changes in DIN concentration, with maximum values occurring during winter overturn (Fig. 1). The extremely low DIN:DRP values in Pyramid Lake are well below plankton biomass (e.g., 7.2, Redfield et al. 1963) and indicate the strong potential for N limitation at all times.

The elemental composition of seston can provide information concerning nutrient deficiencies of phytoplankton growth, and Healey and Hendzel (1980) presented a framework for the interpretation of seston composition in lakes. Based on laboratory and field studies, these authors suggested that the C:N ratio of seston could be used as an indicator of N deficiency of plankton. C:N >14.6 indicates severe N deficiency, while intermediate (8.3–14.6) and low (<8.3) ratios indicate moderate and no deficiency, respectively. Further, they suggested that the N:P ratio of seston could be used to indicate P deficiency. Although P limitation in Pyramid Lake is unlikely due to high DRP concentrations throughout the year, N:P can be used to provide additional evidence for N deficiency. N:P values below those typical for lake plankton (e.g., Redfield ratio of 7.2) support N deficiency in plankton.

Seston elemental composition of Pyramid Lake indicates that N deficiency in phytoplankton varies considerably (Table 1). Although the mean value for C:N was 11.3, indicating moderate N deficiency of plankton, values ranged from 6.0 to 25.3 during 1989–92. An examination of C:N in surface waters for monthly samplings revealed periods of no, moderate, and severe N deficiency during the study (Fig. 1). Similarly, the evidence from seston N:P provides support for varying degrees of N deficiency of phytoplankton. Although mean N:P (7.9, Table 1) was similar to typical values for lake plankton, monthly values were highly variable and indicated periods of both N-deficient (N:P < 7.2) and N-sufficient growth (Fig. 1).

Reuter et al. (1993) presented a seasonal pattern for seston C:N based on the data collected during the first year of our study. For 1990, a period of no deficiency followed winter mixing in January–February. Then, C:N progressively increased to a maximum value in October before decreasing again during cooling of surface waters in the fall. This general seasonal pattern, although not repeated exactly in 1991 and 1992, identified two contrasting periods: winter–spring with sufficient N for growth and summer–fall when phytoplankton growth was N deficient. Clearly, the increased availability of DIN following winter overturn is reflected in the composition of seston during early spring, with C:N values of 6–8 during all years (Fig. 1). The aspect of the 1990 pattern that was not reproduced in all years was the gradual development of severe N deficiency during summer–fall. Although severe N deficiency was restricted to the stratified period, it was not displayed in all years (e.g., 1991).

Natural phytoplankton assemblages from Pyramid Lake showed a dramatic growth response to the addition of N in laboratory experiments. In treatments receiving DIN additions of 20, 50, and 100 µg N·L^{-1} to lake water, there was a significant ($P < 0.05$) increase in algal Chl *a* relative to controls in 68% ($n = 19$), 79% ($n = 19$), and 90% ($n = 10$) of total experiments, respectively (Table 2). The average stimula-

TABLE 2. Final Chl *a* concentrations (mean ± SD) for bioassay experiments with Pyramid Lake surface waters. Values are given for no additions (Con), additions of 20, 50, and 100 μg N·L^{-1} (+N), addition for 20 μg P·L^{-1} (+P), and addition for 50 μg Fe·L^{-1} (+Fe). Experiments were conducted including treatments with simultaneous additions of P (n = 9) or Fe (n = 3) and N. The results from the combined nutrient additions were identical to the corresponding treatment with only N addition. Underlined values are significantly different (Student's t-test) from the control at $P < 0.05$, while values in bold are significant at $P < 0.01$.

Date	Con	+20N	+50N	+100N	+20P	+50Fe
1989						
November	0.33 ± 0.11	**0.74 ± 0.04**	**2.10 ± 0.01**		0.30 ± 0.12	
1990						
February	1.20 ± 0.12	1.21 ± 0.50	1.10 ± 0.08		1.06 ± 0.05	
March	1.60 ± 0.07	**2.53 ± 0.05**	10.11 ± 2.78		1.78 ± 0.39	
April	1.37 ± 0.03	**3.83 ± 0.41**	**9.40 ± 0.66**		1.48 ± 0.10	
May	1.20 ± 0.14	1.56 ± 0.03	1.71 ± 0.19		0.81 ± 0.22	
June	1.18 ± 0.06	**2.99 ± 0.18**	**5.40 ± 0.14**			**0.82 ± 0.10**
July	0.64 ± 0.01	**1.51 ± 0.12**	**3.93 ± 0.47**			0.49 ± 0.06
August	0.54 ± 0.13	**1.85 ± 0.18**	**3.14 ± 0.32**			0.77 ± 0.15
September	3.41 ± 1.60	5.64 ± 0.97	5.77 ± 1.58			6.60 ± 0.09
October	0.77 ± 0.10	1.04 ± 0.08	**1.98 ± 0.25**	**4.27 ± 0.46**		
1991						
February	2.54 ± 0.25	2.24 ± 0.49	2.78 ± 0.56	**1.74 ± 0.26**		2.17 ± 0.40
May	0.80 ± 0.14	**1.47 ± 0.09**	**1.93 ± 0.31**	**2.80 ± 0.21**		0.95 ± 0.06
June	0.54 ± 0.31	0.45 ± 0.08	0.92 ± 0.09	**2.24 ± 0.49**		0.34 ± 0.02
July	0.26 ± 0.02	0.43 ± 0.13	0.66 ± 0.11	**1.47 ± 0.21**	0.25 ± 0.07	0.25 ± 0.04
October	0.69 ± 0.15	1.83 ± 0.57	1.96 ± 0.27	3.75 ± 0.80		0.70 ± 0.10
November	0.37 ± 0.17	0.96 ± 0.07	1.77 ± 0.65	**1.89 ± 0.34**	0.51 ± 0.03	0.35 ± 0.07
1992						
March	0.98 ± 0.22	**2.10 ± 0.25**	1.71 ± 0.10	**1.78 ± 0.19**	0.69 ± 0.02	0.98 ± 0.13
June	0.60 ± 0.10	0.58 ± 0.05	**1.25 ± 0.14**	**1.26 ± 0.11**	0.47 ± 0.05	0.54 ± 0.12
July	1.73 ± 0.14	**3.65 ± 0.10**	**6.77 ± 0.34**	**15.3 ± 1.1**	**1.17 ± 0.18**	1.89 ± 0.14

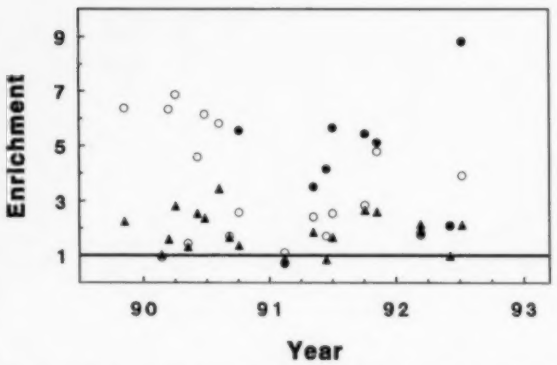

FIG. 2. Enrichment factors for the addition of 20 (▲), 50 (○), and 100 (●) μg N·L^{-1} to Pyramid Lake surface water during 1989–92. The enrichment factor is defined as the final Chl *a* concentration in each treatment divided by the control, with a value of 1.0 indicating no stimulation.

tion of phytoplankton during the study period was striking at 1.9 (n = 19), 3.5 (n = 19), and 4.3 (n = 10) times the controls for the addition of 20, 50, and 100 μg N·L^{-1}, respectively. Additions of 20 μg P·L^{-1} or 50 μg Fe·L^{-1} to lake water did not significantly stimulate phytoplankton on any occasion, although growth was significantly lower on two occasions. Further, the addition of either P (n = 9) or Fe (n = 3) in combination with 20 μg N·L^{-1} in several of the experiments did not enhance the stimulatory effect of adding N alone (data not shown). During 1989–92, the relative growth response of phytoplankton to N addition was often large, especially in treatments with added N of 100 μg·L^{-1}; enrichment factors >5 occurred during all years (Fig. 2). The enrichment factor is defined as the final Chl *a* concentration in each treatment divided by the control, with a value of 1.0 indicating no stimulation of growth relative to the control treatment.

N addition did not lead to a stimulation of phytoplankton growth relative to controls during periods of winter mixing and during a blue-green algal bloom. For bioassay experiments conducted in February of both 1990 and 1991, final Chl *a* concentrations in N addition treatments were similar to the controls, indicating that phytoplankton growth was not limited by N (Table 2). This lack of stimulation during winter mixing can be attributed to the large increase in DIN during winter overturn. In fact, growth in the control treatment during February 1991 was quite large, with a 500% increase in Chl *a* concentration during the experiment (0.48–2.54 μg·L^{-1}). The only other time when N addition did not significantly stimulate phytoplankton growth was in September 1990, when heterocystous blue-green algae comprised a large fraction of the phytoplankton (C.L. Rhodes, unpublished data). In the September 1990 bioassay, growth in the control treatment was >2 μg Chl *a*·L^{-1}, indicating that *N. spumigena* cells were actively growing without the need for nutrient additions. Therefore, a temporary relief for phytoplankton growth from chronically low N availability occurs following overturn in winter and during *N. spumigena* blooms.

Blooms of *N. spumigena* were observed during summer–fall of all three years of the study. In the context of this discussion, we will only use qualitative information about the timing and magnitude of the observed blooms. The

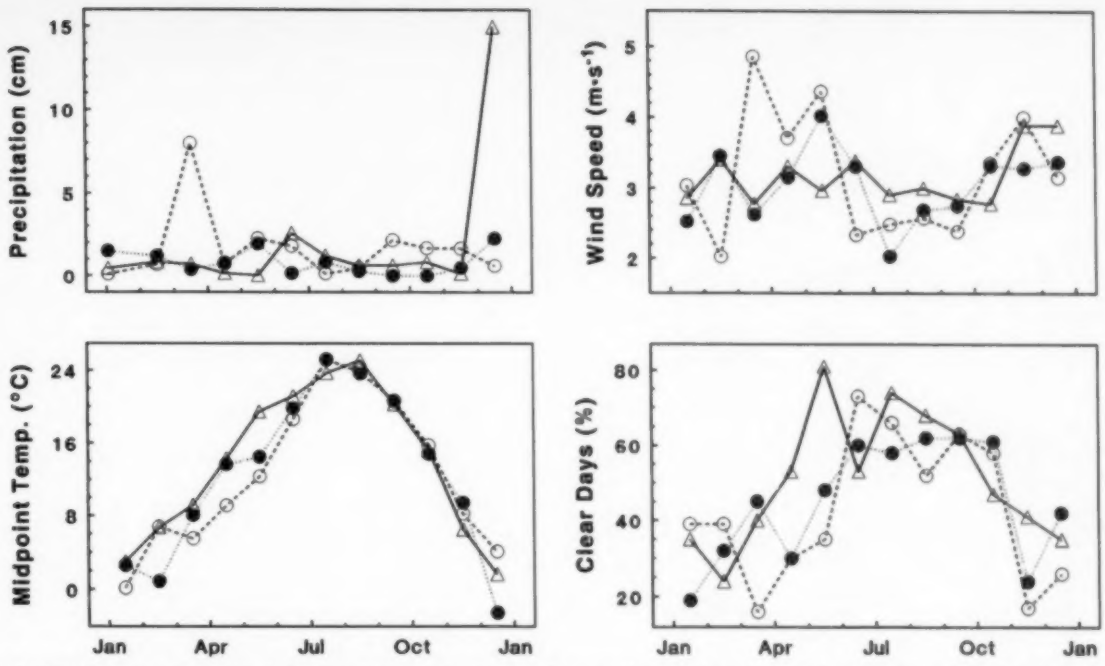

FIG. 3. Precipitation, midpoint air temperature, wind speed, and proportion of clear days derived from weather data recorded at Sutcliffe, Nevada. Values are monthly means for 1990 (●), 1991 (○), and 1992 (△).

timing of the *N. spumigena* bloom varied among years with an early bloom in 1992 (20 July – 21 August) and later blooms in 1991 (20 August – 19 September) and 1990 (4–18 September). The largest bloom during 1990–92 occurred in 1992 when Chl *a* concentration in surface waters increased to 292–549 $\mu g \cdot L^{-1}$ in the upper 1 m of the water column. By comparison, the *N. spumigena* blooms observed during 1990 and 1991 were small relative to the large one in 1992.

Interannual Variations in Indicators

DIN concentration in surface waters during summer stratification was highest during 1991 and lowest in 1992 (Fig. 1). The average for monthly values during May–October 1991 (mean ± SD) was 16.4 ± 4.5 $\mu g \cdot L^{-1}$ compared with 13.2 ± 3.1 and 7.3 ± 2.9 $\mu g \cdot L^{-1}$ for the same period in 1990 and 1992, respectively. A comparison of these average values of DIN concentration during the stratified period revealed that the value in 1992 was significantly (Student's *t*-test: $P < 0.001$; $n = 6$) lower than in 1990 and 1991. Because of similar DRP concentrations in all years, the relative differences in DIN:DRP between years were identical to DIN, indicating the largest potential for N limitation of phytoplankton in 1992 and the lowest in 1991. The potential for N limitation for 1990 indicated by DIN:DRP was intermediate between 1991 and 1992.

The elemental composition of seston indicates that the timing and intensity of N deficiency were also different among years (Fig. 1). For C:N, 1990 and 1992 both exhibited peak values >14.6 during summer–fall, indicating periods of intensely N-deficient growth, with the peak earlier in 1992. Surprisingly, seston C:N was relatively constant during 1991 and indicated only moderate N deficiency. Consistent with general C:N patterns were interannual variations in seston N:P. Although more variable than C:N, variations in seston N:P corroborate several features apparent in the C:N data. N:P supports (1) severe N deficiency in fall 1990, (2) moderate to no N deficiency during June–November 1991, (3) severe N deficiency by July 1992, and (4) no N deficiency during fall 1992.

The results of nutrient enrichment bioassay experiments support a greater availability of DIN during 1991 than in 1990 (Fig. 1 and 2). Clearly, stimulation of phytoplankton growth was much greater in 1990 compared with 1991. In 1990, the stimulation of phytoplankton by the addition of 50 $\mu g\ N \cdot L^{-1}$ was large (e.g., enrichment factor >3) on five of nine occasions compared with only once ($n = 6$) in 1991. Lower stimulation in 1991 supports the hypothesis of greater DIN availability in that year relative to 1990. Conclusions regarding the response of phytoplankton to N addition during 1992 are limited by sparse data, with experiments conducted on only three occasions. On initial inspection, bioassay data for July of 1990 and 1992 indicate a greater response during 1990, contradicting the data from DIN and seston indicators of limitation. However, actual phytoplankton growth relative to controls was higher in the July 1992 experiment (5.1 μg Chl $a \cdot L^{-1}$) than in the comparable one for 1990 (3.3 μg Chl $a \cdot L^{-1}$) (Table 2), consistent with the other indicators of N limitation.

Meteorological Variations

Meteorological data for 1990 indicate that weather variables during that year were generally near the middle of the range displayed for the 1990–92 period of interest (Fig. 3). After a brief cold period in February where the midpoint temperature was 0.9°C, air temperature warmed progressively to

a maximum value of 25.2°C in July. The spring period was generally cloudy (<50% clear days) with moderate morning winds (2.5–3.5 $m \cdot s^{-1}$). Following this cloudy, moderately windy spring period was a relatively sunny, calm summer period with clear days 60% of the time and average morning wind speed of 2.0–2.5 $m \cdot s^{-1}$. In the fall, wind speeds and the number of cloudy days increased again to values similar to the spring period and air temperature decreased. Perhaps, one weather variable not near average values for the lake during 1990 was precipitation, with only 9.8 cm of rainfall for the entire year or 66% of average (Galat et al. 1981), reflecting a regional drought.

Weather conditions during 1991 indicate some important differences from the general patterns described for 1990. Although February 1991 was warm and calm compared with 1990, the spring period in 1991 was cold, cloudy, and windy (Fig. 3). Indeed, monthly averaged midpoint air temperatures during March–May 1991 were 2.2–4.6°C colder than the same period in 1990. Morning wind speeds in spring 1991 were also high (3.7–4.9 $m \cdot s^{-1}$), indicating strong wind-forcing on the surface of the lake. Clearly, early spring in 1991 was stormy during March, with 8.0 cm of precipitation and clear days only 16% of the time. The cold, stormy spring during 1991 was followed by sunny, calm conditions in summer and typical air temperatures. However, surface water temperature during June and July 1991 continued to reflect the cold spring conditions, with an average surface (0–10 m) temperature of 18.2°C during July 1991 compared with 21.9°C in 1990.

Contrasting the cold spring of 1991, weather data for 1992 indicate a warm, clear spring (Fig. 3). During April–June 1992, monthly averaged midpoint air temperature was higher than in 1990, especially in May (4.9°C). Further, a majority of days during April–September 1992 were clear, indicating a large potential for early warming of surface waters. Warm, clear conditions during spring 1992 were accompanied by moderate wind speeds of 2.8–3.4 $m \cdot s^{-1}$. This moderate wind-forcing during 1992 provided sufficient energy to mix warm surface waters down below a depth of 20 m. Indeed, the average water temperature for the 18–22 m strata was 16.2°C in July 1992 compared with 12.1–12.6°C for 1990 and 1991.

Discussion

Comparison of Indicators

The correspondence among the different indicators of N limitation used in this study depended on the degree of N deficiency. While DIN:DRP always indicated the potential for N limitation of phytoplankton growth, seston elemental composition and algal growth response in bioassays indicated periods of sufficient, moderately deficient, and severely deficient growth relative to N availability. During periods of apparent severe N deficiency such as fall 1990, all indicators of potential limitation (except TN:TP) supported severe N limitation of phytoplankton. The good correspondence among indicators during periods of severe N deficiency is contrasted by contradictory information from the different indicators of limitation when apparent N deficiency is only moderate. For example, seston C:N indicated a gradual increase in N deficiency during spring 1990, while bioassay experiments showed a large stimulation of phytoplankton to DIN addition. The results of this study reinforce the need to use multiple indicators of potential limitation in the assessment of the nutrient status of a water body.

The degree of N deficiency of seston indicated by C:N and N:P often showed a poor correspondence during periods when N deficiency was probably moderate. Whereas the variations in C:N demonstrated coherent seasonal patterns, especially in 1990 and 1992, N:P was more variable between successive months (e.g., Fig. 1). A good correspondence between C:N and N:P did exist during periods of intense N deficiency as indicated above. However, information concerning N deficiency was often contradictory between periods of N sufficiency during winter mixing and severely deficient growth during summer causing scatter in the relationship between the seston ratios ($r = 0.26$; $P = 0.1$; $n = 37$).

One factor contributing to a greater variability in N:P relative to C:N in Pyramid Lake is seasonal variations in the contribution of inorganic P phases to PP. Lebo et al. (1993) showed that eolian transport of P-rich dust was a source of P that can elevate PP concentrations along the leeward margins of the lake. The resuspension of bottom sediments during winter deep mixing may also contribute to seston inorganic P during spring (M.E. Lebo, unpublished data). The combined effect of these two sources of particulate matter is to artificially lower seston N:P values during spring periods. It is probable that low N:P values observed during spring periods such as March 1991 (Fig. 1) can be attributed to the input of inorganic PP to the water column. Because the 1989–92 period was during a drought, a large source of particulate matter to the lake, the Truckee River, was not considered in the above discussion. During years of moderate to high inflow, sediment loading to the lake may further reduce seston N:P in surface waters relative to the values observed during 1989–92. A consequence of seston inorganic P is that N:P during periods of high inorganic turbidity should be viewed cautiously.

A second factor contributing to greater variability in N:P relative to C:N is detrital C. The presence of detrital C in the seston of surface waters should reduce the response of C:N to changes in N availability. Indeed, the change in N:P of seston following two periods of severe N deficiency in November 1989 and July 1992 was large (71–155%), while the concomitant changes in C:N were smaller at 25–33%. For both examples, N availability should have increased between monthly samplings due to fall mixing in 1989 and N_2 fixation by *N. spumigena* during 1992. The change in seston C:N and N:P, however, did not signify the same level of increase in N availability. It seems that N:P may be a more responsive indicator of changes in N availability than C:N provided inorganic seston P is low, as is the case during the summer–fall stratified period.

Effects of Climatic Variations

Variations in weather conditions during 1990–92 provide an excellent opportunity to examine how climatic factors affect nutrient availability in Pyramid Lake. Because 1990–92 was a drought period, river DIN loading to the lake was extremely low (M.E. Lebo et al., unpublished data). Further, total DIN in the lake prior to winter mixing of each year was similar among years. The primary differences among years, therefore, should be due to variations in climatic conditions at the lake.

Meteorological forcing in lakes affects nutrient cycling in many ways including the timing of mixing cycles. In

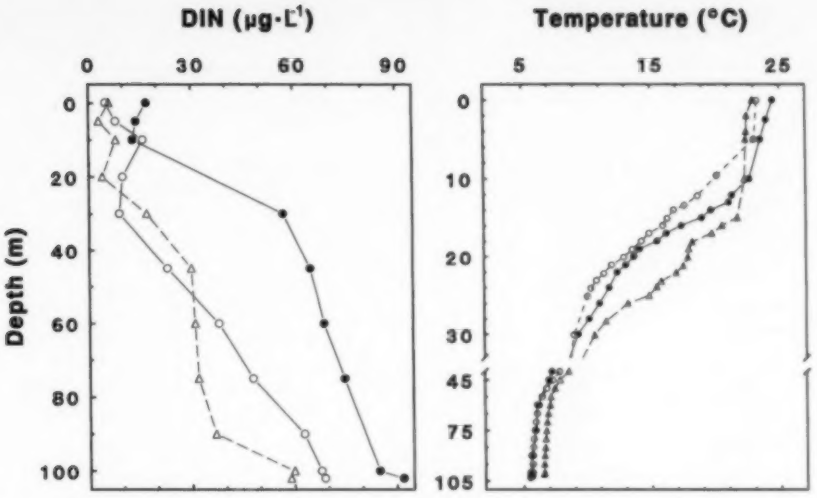

FIG. 4. Spring DIN and August temperature profiles in the center of Pyramid Lake in 1990 (●), 1991 (○), and 1992 (△). DIN profiles are for the monthly sampling following the collapse of the winter–spring bloom with profiles shown for May 1990 and April in both 1991 and 1992. Note the different depth scales on the two plots.

warm, monomictic lakes, such as Pyramid Lake, variations in weather conditions can alter the timing and extent of winter mixing which can have profound effects on annual phytoplankton productivity. For example, Goldman and Jassby (1990) showed that summer phytoplankton production in Lake Tahoe was directly related to the depth of mixing during winter. Although Pyramid Lake generally mixes to the bottom each year, variations in the timing of winter mixing are evident in our 3-yr data set. A good tracer of the onset of winter mixing in Pyramid Lake, as in other N-deficient systems such as Lake Tahoe (Goldman et al. 1989), is DIN concentration, with the period of annual deep mixing corresponding to maximum seasonal DIN concentration in surface waters (Lebo et al. 1992). Three distinct peaks in DIN concentration of surface waters and one diminished peak were observed between October 1989 and December 1992 (Fig. 1), indicating that deep mixing occurred in February 1990, January 1991, January 1992, and December 1992.

Variations in air temperature and wind speed can explain relative differences in observed timing of deep mixing during 1990–92. The earliest time of mixing was in late December 1992 during a period of winter storms with persistently strong winds (Fig. 3). The influence of wind speed on deep mixing also was apparent during winter 1991–92 when maximum DIN occurred in January 1992 following strong winds in November 1991. In fact, the diminished DIN peak in January 1992 indicates that deep mixing probably occurred a couple of weeks prior to our monthly sampling. In contrast with these two cases of strong wind-forcing, weak wind-forcing during winter 1989–90 can explain the delay in deep mixing until February 1990; monthly averaged morning wind speeds during November 1989 – January 1990 were only 1.8–2.6 $m \cdot s^{-1}$ compared with 3.2–4.0 $m \cdot s^{-1}$ during fall of other years. The importance of air temperature on the timing of mixing is evident in winter 1990–91 when cold temperatures during December 1990 contributed to overturn by January 1991.

The timing of winter mixing affects seasonal phytoplankton production in Pyramid Lake by altering the timing of increased DIN concentration in surface waters. Because the winter–spring diatom bloom in Pyramid Lake is fueled by DIN mixed into surface waters during deep mixing (Hamilton-Galat and Galat 1983; Lebo et al. 1992), variations in the timing of winter mixing cause concomitant variations in the timing of the bloom. Indeed, maximum surface water Chl *a* concentrations during 1991 and 1992 occurred in March and late February, respectively, compared with April during the winter–spring bloom in 1990.

The timing of the winter–spring bloom in Pyramid Lake has a profound effect on the stripping of DIN from the water column. During years of early mixing, the diatom bloom period occurs entirely during the unstratified period, while later blooms peak after the onset of thermal stratification. The result of the continual mixing of DIN into surface waters during early blooms is the stripping of a large portion of DIN from the entire water column and not just the euphotic zone (Fig. 4). Clearly, the early blooms during 1991 and 1992 removed a greater proportion of total DIN (approximately 67%) from the total water column than in 1990 (20%); during the early blooms, volume-weighted average DIN was reduced to <20 $\mu g \cdot L^{-1}$ from a starting concentration of 55–60 $\mu g \cdot L^{-1}$. A consequence of the efficient stripping of DIN during early blooms is decreased DIN concentrations following the bloom (April–May) leading to a greater likelihood of severe N deficiency during summer–fall stratification.

The potential for spring–summer N deficiency following the spring bloom period, however, can be modified by the magnitude of wind-forcing during late spring. Generally, strong winds during partial stratification in spring promote upwelling of hypolimnetic waters to the surface along leeward margins, thereby increasing N availability. Lebo et al. (1993) discussed the potential for upwelling in Pyramid Lake during 1986–92 based on morning wind speed data and temperature profiles. The year with the greatest poten-

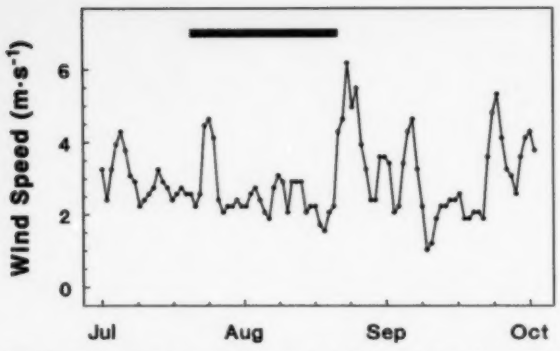

FIG. 5. Daily morning wind speed, 1 July – 1 October 1992. Values are three-point moving averages of daily values. The overbar indicates the period for which N_2 fixation by *N. spumigena* was detected in laboratory assays (C.L. Rhodes, unpublished data).

tial for upwelling during that period was 1991, with four probable upwelling events in May alone. It is probable that the increase in the DIN concentration of surface waters in the center of the lake during May–July 1991 (Fig. 1) is a consequence of frequent upwelling events during that period. Upwelling during 1991 may also have partially contributed to the cooler water temperatures.

Variations in weather conditions during spring–summer of each year contributed to differential warming of surface waters. Although maximum surface water (0–20 m) temperatures were similar in all three years, the date on which surface waters were approximately 20°C or higher varied by over 1 mo. The extremes during our study period are represented by warm surface waters ($T \approx 20$°C) in June 1992 but not until August 1991. The difference in solar heating of surface water among years is clearly evident in the temperature–depth distributions in the center of the lake during August of each year (Fig. 4). Although temperature at the surface was similar among years (23–25°C), warm waters extended to a greater depth in summer 1992 than in the previous two years. Further, the year with the least storage of heat in surface waters (e.g., volume of warm water) was 1991.

There are three climatic factors contributing to interannual differences in the timing and total heat storage during spring warming of surface waters: (1) air temperature, (2) number of cloudy days, and (3) wind speed. The observed differences in the temperature profiles during August of 1990, 1991, and 1992 (Fig. 4) can be explained by variations in these three climatic variables. During 1992, the spring period was warm, calm, and sunny leading to the early warming of surface waters and a large storage of heat; surface waters in August 1992 were >20°C down to a depth of 17 m. The opposite was true during a cold, cloudy spring period (1991) when surface warming to 20°C was delayed until midsummer and these warm surface waters only extended down to a depth of approximately 10 m. In addition, strong sustained winds during cold, cloudy spring periods such as in 1991 also promote upwelling of cold hypolimnetic waters which probably also contributes to delayed warming of surface waters.

One ecological significance of warm surface waters in Pyramid Lake is as a potential controlling factor for the timing of *N. spumigena* blooms during summer–fall. Water temperature has been suggested to be an important factor controlling blue-green algae biomass in lakes (Tilman et al. 1986; McQueen and Lean 1987), and previous studies at Pyramid Lake suggest that warm surface waters may contribute to *N. spumigena* bloom development (Galat and Verdin 1988, 1989; Galat et al. 1990). Based on our observations, we hypothesize that warm surface water temperatures are an important prerequisite that defines the potential window in which a bloom may develop. However, water column stability and turbulence probably control bloom formation and collapse (e.g., Paerl et al. 1981; Ernst 1990).

Morning wind data from summer 1992 indicates that the strength of wind-forcing during periods of warm surface waters may play an integral role in *N. spumigena* bloom formation and collapse (Fig. 5). During the period shown, a large *N. spumigena* bloom formed in the lake, with N_2-fixation detected between 20 July and 21 August. There are two important features in the morning wind speed data during the 1992 bloom period. These are a generally calm period in which the bloom developed and a large wind event in the middle of August that caused the bloom to collapse. It is also possible, once surface waters are warm, that a windy period is necessary to resuspend littoral bottom sediments, thereby providing a seed population of vegetative cells or akinetes. When wind data from other years are examined, it is interesting to note that the same general features described for 1992 also characterize the wind records at Sutcliffe for the three other years during 1986–92 with recorded *N. spumigena* blooms.

The similarity among wind patterns during these four years provides support for a wind-forcing mechanism controlling *N. spumigena* formation and collapse. After surface waters warm, bloom proliferation requires a prolonged period of low to moderate winds during which cells grow. The moderately strong wind event that was recorded during the early part of the 1992 bloom (24 July) indicates that an extremely calm period is not necessary for bloom development. We hypothesize that the duration and windiness during this growth period dictate the relative magnitude of the bloom for the year, rather than any chemical factors. The growth period for the bloom is curtailed by strong winds that are sustained over several days. Perhaps, it is no coincidence that the collapse of the last four recorded blooms occurred during periods of sustained strong winds. A more extensive examination of climatic control of *N. spumigena* production in Pyramid Lake is currently being conducted in which we explore this wind-forcing mechanism more thoroughly.

Another variable related indirectly to climate is river inflow. Throughout this paper, we have considered interannual variations in nutrient limitation at Pyramid Lake and how those variations are related to weather conditions. However, river inflow will also modify these relationships in several important ways. First, the Truckee River can be an important source of DIN to the lake during spring (Galat 1990) which may even forestall intense limitation in years with high inflow. A second way in which high Truckee River inflow can affect N availability is by causing salinity stratification in surface waters (Galat and Verdin 1988). This salinity stratification of the water column will generally precede the spring heating cycle for surface waters and facilitate early stratification. Early stratification of the water

column will have two main effects: (1) reduced mixing that will decrease DIN availability and (2) early warming of surface waters that will further reduce mixing. Both of these effects will contribute to greater N deficiency in the early summer. The early warming of surface waters will also extend the "window" of warm surface waters providing a greater potential for large *N. spumigena* blooms during years with high inflow.

Conclusions

The results of this study indicate that weather patterns can have a pronounced effect on the degree of nutrient limitation in a lake. Although N in Pyramid Lake was always the nutrient most limiting to phytoplankton, regional climatic variations among years affected both the timing and overall magnitude of limitation in different years. Climatic variations affected the timing of winter mixing, the potential for spring upwelling, the timing of thermal stratification, and the formation and collapse of the summer–fall blue-green algal bloom. These limnological variations among years induced by climate had a marked impact on nutrient cycling and availability.

An important consequence of interannual variability of nutrient limitation induced by climate is its potential effect on evaluation of water quality trends. The ability of monitoring programs, either national or for individual lakes, to ascertain trends will be strongly affected by regional climatic variations. Therefore, it is essential that potential climatic variations be addressed in the assessment of nutrient limitation and long-term trends in lakes.

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Allozymic Variation and Species Diversity in North American Bosminidae

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North American members of the genus *Bosmina* show marked morphological variation and integradation, which have been attributed to the occurrence of both phenotypic plasticity and occasional hybridization between a small number of species. We employed allozyme analysis on 72 bosminid populations from 62 North American habitats to ascertain the validity of species boundaries and to verify the occurrence of hybridization. Results showed that levels of genetic variability (15.8% polymorphic loci, 10% individual heterozygosities) in bosminids were comparable with those reported in other zooplankton groups. Significant Hardy–Weinberg deviations were detected at 38% of polymorphic loci and were usually due to heterozygote deficiency. No clear evidence of interspecific hybridization was obtained, although some cases of heterozygote excess require further investigation. While prior morphological studies indicated the occurrence of only six species, the allozyme data revealed the presence of eight assemblages showing allelic substitutions at one or more loci. Each assemblage corresponded to a different species belonging to one of four subgenera (*Bosmina*, *Eubosmina*, *Neobosmina*, or *Sinobosmina*). Within two assemblages, individuals could be further partitioned into two morphologically distinct, but genetically similar species, leading to the recognition of 10 species in this study.

Les membres nord-américains du genre *Bosmina* témoignent d'une variation morphologique et d'un chevauchement marqués, qui ont été attribués à leur plasticité phénotypique et à l'hybridation occasionnelle entre un petit nombre d'espèces. Nous avons procédé à l'analyse des allozymes de 72 populations de bosminidés qui provenaient de 62 habitats d'Amérique du Nord afin d'estimer la validité des limites des espèces et de vérifier la fréquence d'apparition de l'hybridation. Les résultats montrent que les niveaux de variabilité génétique (15,8 % de polymorphisme chromosomique, 10 % d'hétérozygotie dans la composition génétique de chaque individu) chez les bosminidés sont comparables à ceux rapportés pour d'autres groupes zooplanctoniques. Des écarts significatifs de fréquence selon la loi Hardy–Weinberg ont été détectés à 38 % des loci où il y a polymorphisme; ils sont ordinairement attribuables à une hétérozygotie inférieure à ce qui est attendu. On n'a pas obtenu d'indice certain d'une hybridation interspécifique même si, parfois, une surabondance d'hétérozygotes appelle un complément de recherche. Bien que des études morphologiques antérieures aient indiqué l'existence de seulement six espèces, les données sur les allozymes indiquent la présence de huit groupes où on observe des substitutions d'allèles à un locus ou plus. Chaque groupe correspond à une espèce différente qui appartient à un de quatre sous-genres (*Bosmina*, *Eubosmina*, *Neobosmina* ou *Sinobosmina*). Dans deux groupes, les sujets pouvaient être aussi divisés en deux espèces morphologiquement distinctes, mais génétiquement similaires; de la sorte, notre étude nous amène à identifier 10 espèces.

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Although allopatric speciation is perhaps the most common speciation mechanism, Mayr (1963) concluded that it was unlikely in freshwater zooplankton because he presumed species to be essentially panmictic across their broad distributions. For example, in the freshwater Cladocera, resting eggs allow for the dispersal of taxa, and are assumed to support pervasive gene flow. If this is true, then single species may show broad geographic distributions with little genetic divergence among their component populations. The assumptions of low species diversity and cosmopolitanism in the cladoceran crustaceans have rarely been critically appraised. However, detailed morphological and genetic work on selected groups of zooplankton over the past decade has revealed that earlier taxonomic studies seriously underestimated species diversity (Hebert 1977; Frey 1982; Lieder 1983b). It is now recognized that there is a need for similar critical appraisal on a broader range of zooplankton groups.

Members of the Bosminidae are a dominant component of the freshwater cladoceran community. In North America, it is believed that there a few species of bosminids exhibiting widespread distributions and extreme morphological variability. The two taxonomic keys now available for the North American fauna are based on morphology, and although they are in general agreement concerning the number of species, they conflict in ranking of the major taxonomic groups. Deevey and Deevey (1971) recognized two genera, *Bosmina* and *Eubosmina*, consisting of one and four species, respectively. Lieder (1983b) assigned these same five species to three different subgenera (*Bosmina*, *Eubosmina*, and *Neobosmina*) of the genus *Bosmina*. Although members of a

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TABLE 1. Locations and abbreviations of habitats sampled in North America. For a detailed description of locations, see De Melo (1993).

Abbrev.	Sample site	Lat. (N)	Long. (W)
LE-1	Leamington	41.56	82.34
LE-2	Rondeau	42.15	81.45
LH-1	Lion's Head		
	Site 1	45.00	81.44
	Site 2	45.07	81.15
LO-1	Bay of Quinte, Belleville[a]		
	Station B	44.09	77.20
	Station HB	44.05	77.04
	Station N	44.11	77.03
LO-2	Midlake, Station 41[a]	43.43	78.01
LO-3	Hamilton Harbour	43.12	79.45
LO-4	Kingston	44.12	76.33
LS-1	Ashland	46.35	90.50
QUE-1	Pond, Lac Etchemin	46.20	70.30
NB-1	Gibson Lake	45.10	67.05
NEWF-1	Pond, Arnold's Cove	47.35	54.00
NEWF-2	Pond, Arnold's Cove	47.30	53.56
NEWF-3	Pond, Cornerbrook	48.50	57.45
NEWF-4	Pond, Cornerbrook	48.45	57.45
NEWF-5	Pond, Stephenville	48.25	58.20
NEWF-6	Pond, Cornerbrook	48.35	58.00
NEWF-7	Pond, Cornerbrook	48.42	57.50
NEWF-8	Pond, Blandford	48.00	54.00
NEWF-9	Pond, Little Harbour	47.35	53.52
NEWF-10	Pond, Mount Pearl	47.25	53.05
NWT-1	Pond, Iqaluit	64.00	70.00
ONT-1	Lake Belwood	43.60	80.20
ONT-2	Lake Conestogo	43.42	80.44
ONT-3	Echo Lake	45.11	79.04
ONT-4	Lake Eugenie	44.19	80.30
ONT-5	Gordon Pittock Reservoir	43.10	80.45
ONT-6	Guelph Lake	43.36	80.15
ONT-7	Lake Mijnemungshing	47.42	84.42
ONT-8	Lake Opeongo	45.42	78.23
ONT-9	Plastic Lake	45.11	78.50
ONT-10	Rice Lake	44.05	78.19
ONT-11	Lake Scugog	44.06	78.57
ONT-12	Sherborne Lake	45.11	78.47
ONT-13	Lake Simcoe	44.24	79.30
ONT-14	Stony Lake	44.33	78.06
ONT-15	Sturgeon Lake	44.28	78.43
ONT-16	Wabikowa Creek Lake	48.47	85.45
ONT-17	White Lake	48.47	85.37
ONT-18	Wildwood Reservoir	43.15	81.05
ONT-19	Wren Lake	45.11	78.52
ARK-1	Pond, Princeton	34.08	92.35
CAL-1	Hume Lake	36.47	118.54
CONN-1	Columbia Lake	41.43	72.20
CONN-2	Lake, Mansfield Four Corners	41.48	72.15
IND-1	James Lake	41.19	85.43
IND-2	Kuhn Lake	41.17	84.43
IND-3	Lake Tippecanoe	41.19	85.45
IND-4	Waldron Lake	41.29	85.25
IND-5	Lake Wabee	41.23	85.49
IND-6	Lake Webster	41.19	85.40
IND-7	Winona Lake	41.13	85.49
MA-1	Togue Pond	46.56	68.53
MASS-1	Long Pond	41.30	70.10
MASS-2	Pond, Truro	41.59	70.05
MICH-1	Austin Lake	42.10	85.33
MICH-2	Eagle Lake	42.19	85.19
MICH-3	Lower Crooked Lake	42.12	85.42
MICH-4	White Lake	48.50	85.40
NY-1	Canopus Lake	41.27	73.50
NY-2	Lake Oneida	43.12	75.55
NY-3	Payne Lake	44.16	75.38
NY-4	Salmon River Reservoir	43.32	75.55
RI-1	Lake, West Gloucester	41.52	71.45
TEX-1	Pond, Amarillo	35.20	102.00
WIS-1	Lake Wingra	43.03	89.25
MEX-1	Presa Santa Ana	20.03	97.30

[a]Canada Centre for Inland Waters (CCIW) sampling sites.

fourth subgenus, *Sinobosmina*, have been reported only from Asia (Lieder 1983b), an undescribed sinobosminid has been known from numerous locations in North America for at least two decades (U. Lieder, Berlin, Germany, and V. Korinek, Charles University, Prague, personal communication). Despite past work, the identification of North American bosminids remains a frustrating task, reflecting the paucity of morphological characters that are unaffected by environmental conditions and that can be reliably used for taxonomic diagnosis. As well, there is evidence that taxonomic confusion may be enhanced by interspecific hybridization. Lieder (1983a, 1991) has suggested that hybridization has been responsible for generating most of the phenotypic diversity in European forms, and probably also in North American taxa. Although genetic studies have not been employed to verify this hypothesis in bosminids, allozyme studies on other groups of cladocerans have revealed an unusual prevalence of F_1 hybrids. For example, Hebert et al. (1989) reported extensive hybridization between *Daphnia pulex* and *D. pulicaria* in North American ponds, while Taylor and Hebert (1992) showed that F_1 hybrids between *D. galeata mendotae* and *D. rosea* constituted more than 30% of the daphniids present in Indiana lakes.

The lack of good morphological characters and the potential importance of hybridization suggest that morphological studies alone cannot clarify taxonomic problems in the Bosminidae. The ability to recognize hybridization events, as well as sibling species, using allozyme analysis has been exploited to advance the understanding of species boundaries in several zooplankton groups (e.g., *Daphnia*: Hebert 1977; Taylor and Hebert 1992; *Leptodiaptomus*: Boileau and Hebert 1988; *Simocephalus*: Hann and Hebert 1986). Prior genetic work on *Bosmina longirostris* from Lake Washington resolved only a single allyzome locus, but this was sufficient to distinguish two sympatric species (Manning et al. 1978). Similar results were obtained for the same species collected from Lake Travis, Texas (Brock 1980).

TABLE 2. Genotypes at individual allozyme loci observed in populations of North American bosminids. Genotypes are listed in order of abundance, but only those with >5% occurrence in a taxon are shown for polymorphic loci. Eight genetic assemblages were recognized. The subgeneric membership of each assemblage is indicated together with a letter code for those cases in which two or more taxa were recognized from a particular subgenus. Diagnostic differences among assemblages of a single subgenus are in bold italic. Numbers refer to alleles of different mobilities. Multilocus genotypes are not shown.

Genetic assemblage	Taxon	Enzyme							
		AAT	AO	APK	FUM	GPI	LDH	MDH	PGM
1 A	*Bosmina s. s.*	34 33	33	33	13 11	33	44	22	35
1 B	*Sinobosmina A*	33	33	33	11 13	33 23	***44***	***22***	33 13 35
2	*Sinobosmina B*[a]	33 23 22	33	33	11	33 22	***33***	***33***	33 35 55 13
3	*Neobosmina A*	22	***33***	33	***33***	33	33	***22***	***66***
4	*Neobosmina B*	44	***22***	33	***11***	33	33	***11***	***22***
5	*Neobosmina C*	22 24 44	***33***	33	***22***	33	33	***22***	***66*** ***67***
6 A	*Eubosmina A*	33	***11***	22	11	44	33 23 13	22	***22*** ***23***
6 B	*E. coregoni*	22 23 33	***11***	22	11	44	33	22	***33*** ***35*** ***13***
7	*Eubosmina B*	33	***22***	22	11	44	33	12	***33***
8	*Eubosmina C*	22	***22***	22	11	44	33	22	***44*** ***45*** ***55***

[a]Individuals heterozygous for the two GPI alleles were also observed, but in low frequency.

If one defines species as reproductively isolated units, it follows that fixed electrophoretic differences between sympatric individuals indicate a lack of interbreeding (Richardson et al. 1986) and, hence, the presence of two or more species. In cases of allopatric populations, the extent of genetic divergence between populations can also be used as a reliable indicator of species boundaries (Richardson et al. 1986). We examined patterns of genetic variation in 72 populations of the genus *Bosmina* (sensu LIEDER) collected from 62 lakes and ponds across North America, with the goal of assessing both local species richness and the total number of species at these sites.

Materials and Methods

Collections

Samples of *Bosmina* were collected from 45 lakes and 17 ponds between May 1990 and September 1992 (Table 1). Only a single site was sampled from each habitat, except in three of the Great Lakes, where six sites were sampled in Lake Ontario and two sites in each of Lakes Erie and Huron. Samples were ordinarily collected either by vertical haul at one site within a lake or by throw net in a pond and sorted within 12 h of collection. Individuals were either flash frozen in liquid nitrogen for electrophoresis at a later date or immediately electrophoresed.

Electrophoresis

The allozyme phenotypes of individual bosminids were determined using modifications (De Melo 1993) of standard cellulose–acetate electrophoresis (CAGE) methodologies (Hebert and Beaton 1989). CAGE allowed for the resolution of a minimum of three enzymes for adults of the smallest species (0.3 mm) and eight enzymes for adults of the largest species (0.8 mm). Since an overview of total variation within each population was desired, data from different individuals within a single population were pooled when each organism was too small to be screened for all enzymes. Each population was screened for variation at eight enzymes: aldehyde oxidase (AO), aspartate aminotransferase (AAT), arginine phosphokinase (APK), fumarase (FUM), glucose phosphate isomerase (GPI), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and phosphoglucomutase (PGM). Complicated banding patterns for AO did not permit for the distinction of heterozygotes from homozygotes, but data for this locus were retained with individuals scored as homozygotes because large mobility differences were noted among populations of putative taxa. For the remaining loci,

banding patterns were interpreted to be under the control of single loci, as the observed heterozygote patterns conformed to those expected from the quaternary structure of the enzyme (Ward 1977). Alleles were identified relative to a standard and scored numerically according to increasing anodal mobility of their allozyme products (i.e., allele 1 corresponds to the allozyme with the slowest mobility). Allozymic data collected from multiple sites within a lake for a particular taxon were pooled if no significant heterogeneity was observed (heterogeneity chi-square χ^2 test, Workman and Niswander 1970) at a significant level of $p = 0.05$.

Population Analyses

Specimens from each habitat were assigned to one or more subgenera based on morphology of the lateral head pore and position of sutures on the mucrone (Lieder 1983b). When individuals of the same subgenus from a specific habitat showed allele substitutions at one or more loci, they were subsequently subdivided into genetic groups.

All statistical analyses of allozyme data were conducted using BIOSYS-1 (Swofford and Selander 1981). A matrix of Nei's (1987) unbiased genetic distance was calculated for all samples to estimate the extent of genetic divergence among populations. This genetic distance matrix was subsequently used to construct an unweighted pair-group matrix analysis (UPGMA) dendrogram to depict genetic relationships among the populations.

Deviations of genotypic frequencies at each variable locus from those expected under Hardy–Weinberg equilibrium were determined by χ^2 analysis using Levene's correction for small sample sizes. In cases where an expected genotypic class was less than one, data were pooled. To assess the significance of the χ^2 tests, the sequential Bonferroni technique was applied separately to each derived probability. This method of a posteriori table-wide significance testing corrects for the occurrence of Type I error (Rice 1989). The number of alleles per locus, average heterozygosity, and the percentage polymorphic loci (using 0.95 criterion) were calculated by direct count.

For all samples, an analysis of the proportion of species detected in relation to the number of sites sampled and the number of loci examined was conducted using the combinatorial method described in Hebert et al. (1988).

Results

Genetic Diversity in Subgenera and Their Component Taxa

Morphological analysis led to the recognition of individuals belonging to each of the four subgenera (Lieder 1983b) of *Bosmina*. Electrophoresis detected the occurrence of eight genetic assemblages within these subgenera based on allelic substitutions at one or more loci (Table 2), with each assemblage presumed to correspond to a different species. However, two assemblages (1 and 6) each consisted of two morphologically distinct species (De Melo and Hebert 1994) that failed to show any fixed differences at the loci screened. Therefore, a total of 10 putative species of bosminids were recognized from the 62 lake and pond habitats.

The sole population of *Bosmina s. s.* was genetically similar to individuals of *Sinobosmina* sp. *A*, but the position of the lateral head pore was distinct in these two groups. Only the presence of the AAT^4 allele and the higher frequency of both the PGM^5 and FUM^3 alleles in *Bosmina* s. s. distinguished this taxon allozymically from *Sinobosmina* sp. *A* (Table 2). The two species of *Sinobosmina* showed fixed differences at two enzyme loci, MDH and LDH. Moreover, two alleles at the FUM locus (FUM^3 and the rare FUM^2) were only detected in members of *Sinobosmina* sp. *A*, while AAT^2 was detected only in members of *Sinobosmina* sp. *B*. The subgenus *Neobosmina* consisted of three taxa that were readily distinguished on genetic criteria. Species *A* and *C* showed a fixed difference at the FUM locus, while species *B* was extremely divergent showing fixation of alternate alleles at four loci (AO, FUM, MDH, and PGM). All four *Eubosmina* taxa were readily discriminated from other bosminids by their possession of unique alleles at APK and GPI. Three of these species possessed a mucro, while *E. coregoni* lacked one. Despite the morphological difference (presence/absence of a mucro) between *E. coregoni* and *Eubosmina* sp. *A*, individuals of these taxa failed to show any allozyme substitutions. However, members of *Eubosmina* sp. *A* did possess two unique alleles (LDH^1 and LDH^2) and were also nearly fixed for the PGM^2 allele, which was absent in *E. coregoni*. *Eubosmina* sp. *B* was identified by its near fixation for both the heterozygous genotype $MDH^{1/2}$ and the AO^2 allele. The AO^2 allele was also found in populations of *Eubosmina* sp. *C*, but this species possessed unique PGM alleles (4 and 5).

The UPGMA dendrogram of genetic distances (*D*) clearly shows five major branches, as well as the separation of nine of the 10 species by genetic distances of 0.2 or greater (Fig. 1). The exceptional species pair (*Sinobosmina* sp. *A* and *Bosmina* s. s.) showed a *D* of 0.09. All four *Eubosmina* species formed a single cluster, while *Neobosmina* species were found in two different branches, with one branch containing taxon *B* and the other containing the remaining two taxa. The two species assigned to the subgenus *Sinobosmina* were assigned to different branches, with one of these branches including *Bosmina s. s.*

Intraspecific genetic distances were typically low (Fig. 1), although divergence linked to occupancy of lake versus pond habitats was noted for several taxa (Fig. 1; Table 3). The most marked example of such divergence was noted in *Sinobosmina* sp. *B*. The AAT^2 allele was present only in pond populations of this species whereas GPI^3, GPI^4, and PGM^6 were found only in lake samples. Pond populations of *Eubosmina* sp. *A* were fixed for AAT^2 and possessed the MDH^1 and PGM^3 alleles whereas lake populations showed a different suite of alleles at these loci. Both populations of *Eubosmina* sp. *C* from ponds possessed allele 5 at PGM, although this allele was not detected in two lake populations. Members of *Neobosmina* sp. *C* also displayed allelic differences between pond and lake populations, but as only one population from each habitat was obtained, it is unclear if these differences were habitat specific.

Genetic Characteristics of Taxa

The mean number of alleles per locus was similar for species in each of the four subgenera (Table 4). The percentage of polymorphic loci ranged from an average of 5.6% in the subgenus *Neobosmina* to 19.8% in the subgenus *Sinobosmina*, although the sole population of *Bosmina s. s.* was polymorphic at 50% of its loci. Individuals of this species also showed a heterozygosity value (42%) seven times higher

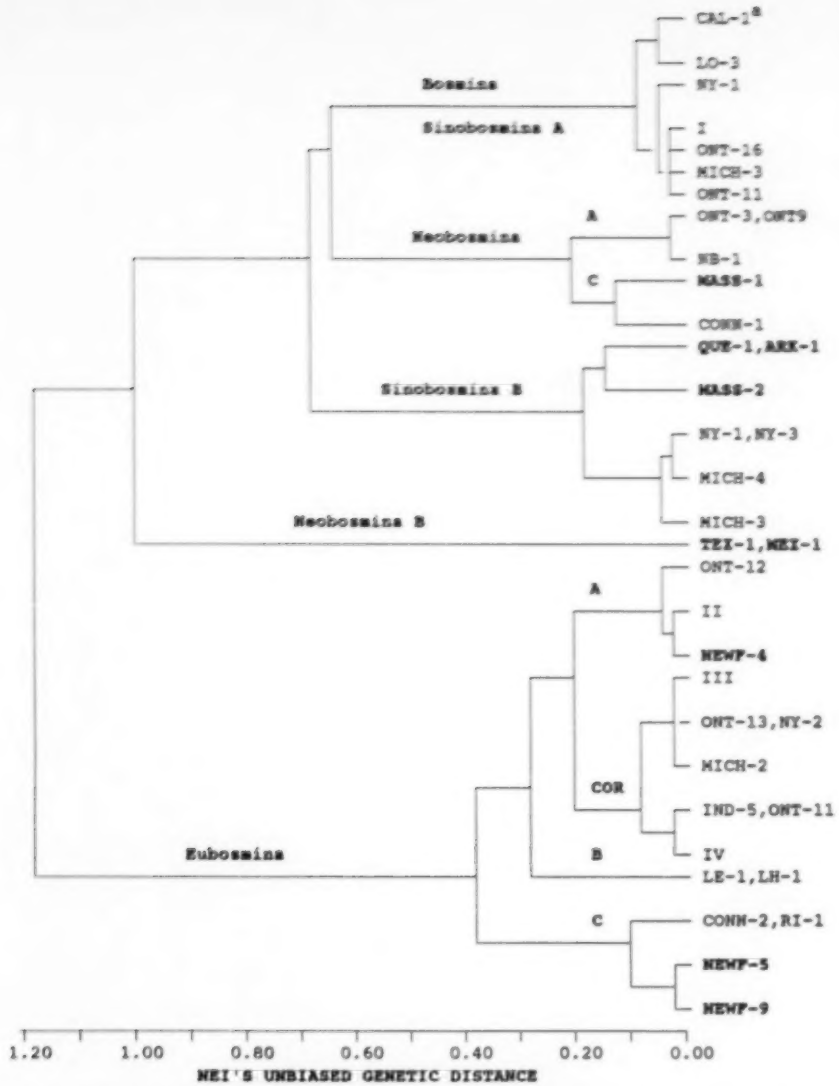

FIG. 1. UPGMA dendrogram of Nei's unbiased genetic distance for 10 putative taxa of North American bosminids. Pond populations are shown in bold in the figure and below. Sample abbreviations are given in Table 1 except the following: a, sole population of subgenus *Bosmina*. Four groups of allozymically identical populations are shown in Roman numerals and include the following populations: I, MICH-1, MICH-4, LS-1, LO (LO-1 and LO-4 data pooled), ONT-17, LE (LE-1 and LE-2 data pooled); II, ONT-7, 8, 19, **NEWF-1–3, 6–8, 10, NWT-1, MA-1**; III, IND-1–3, 6, 7, LO-2; IV, IND-4, ONT-1, 4, 5, 6, 10, 14, 15, 18, NY-4, LO-1, 3, LE-2, WIS-1. Cophenetic correlation = 0.94.

than the average for other species. Only *Neobosmina* sp. *B* individuals were found to lack variation.

Significant deviations from Hardy–Weinberg equilibrium were noted for 33 of 86 tests and were attributable more often to heterozygote deficiency than excess, but the pattern of deviations varied among taxa. The high level of heterozygosity in the sole population of *Bosmina s. s.* was linked to significant heterozygote excesses at three of four loci. Members of *Eubosmina* sp. *B* also showed a consistent heterozygote excess due to its near fixation for a heterozygous genotype at its only polymorphic locus. By contrast, Hardy–Weinberg deviations in populations of *Eubosmina* sp. *A* and *E. coregoni* were usually due to heterozygote deficiencies.

Taxon Diversity

All 17 ponds contained only a single species of bosminid, but two species were found in 14 of 45 lakes and three species were found in three lakes. Members of more than one subgenus were represented in 12 of the 17 mixed-species

TABLE 3. Average allele frequencies in populations of four bosminid species from lake and pond habitats. Numbers refer to alleles coding for allozymes of different mobilities. Alleles detected in only one habitat type are in bold italic.

Taxon	Locus	Allele	Habitat	
			Pond	Lake
Sinobosmina sp. *B*	AAT	***2***	0.40	—
		3	0.60	1.00
	GPI	1	0.08	0.04
		2	0.92	0.07
		3	—	0.84
		4	—	0.05
	PGM	1	0.05	0.08
		3	0.25	0.39
		5	0.70	0.49
		6	—	0.04
Eubosmina sp. *A*	AAT	***1***	—	0.05
		2	1.00	—
		3	—	0.95
	MDH	***1***	0.03	—
		2	0.97	1.00
	PGM	2	0.94	1.00
		3	0.06	—
Eubosmina sp. *C*	PGM	4	0.19	1.00
		5	0.81	—
Neobosmina sp. *C*	AAT	***2***	—	0.74
		4	1.00	0.26
	PGM	5	1.00	0.50
		7	—	0.50

lakes. Examination of the relationship between the number of sites sampled (Fig. 2A) and the number of species detected suggested than an asymptote in species richness had been reached, but the relationship between number of enzymes examined and species detected (Fig. 2B) showed only an approach to an asymptote.

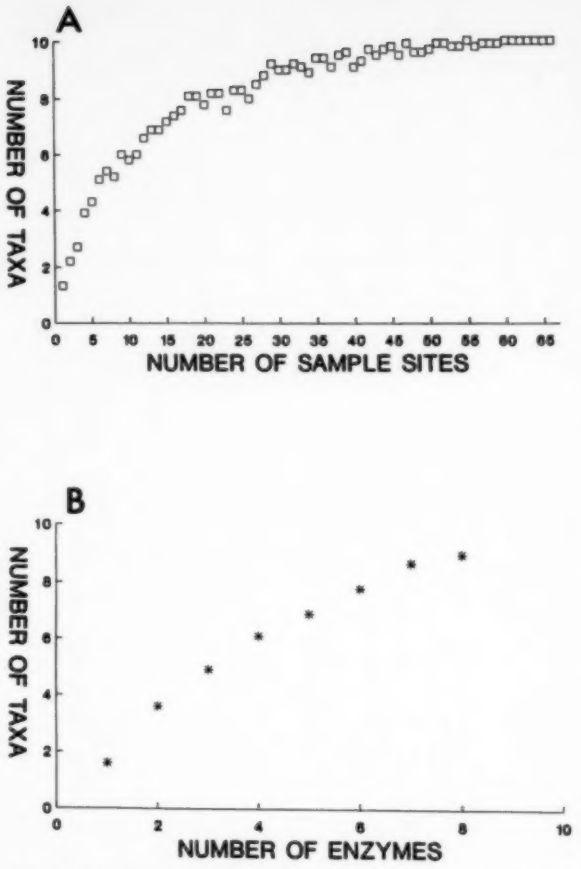

FIG. 2. Saturation curve for the number of taxa recognized in relation to (A) the number of sites sampled and (B) the number of enzymes examined.

Discussion

The use of allozyme analysis in the resolution of taxonomic difficulties has gained widespread acceptance (Ayala 1983; Richardson et al. 1986). Recognition of reproductive isolation (and hence, species boundaries) is most objective when taxa co-occur. In this situation the absence of heterozygotes at a single allozyme locus is a reliable indicator of reproductive isolation. In cases of allopatry, decisions based on genetic analysis are less clear cut. However, prior studies have established that the extent of genetic divergence is ordinarily a reliable indicator of taxonomic status (Avise 1975; Ayala 1983; Thorpe 1983), although there are exceptions to this rule (Avise et al. 1975; Tauber and Tauber 1977; Beardmore and Abreu-Grobois 1983; Johnson et al. 1984, 1986; Snell 1991). It is, however, generally accepted that taxa showing both morphological differences and genetic evidence of reproductive isolation should be recognized as distinct species.

This study of allozyme diversity in *Bosmina* from 62 North American lakes and ponds indicated the presence of eight genetically distinct groups of populations. Supplemental morphological studies have shown that two of these groups were an amalgam of two phenotypically distinct taxa (De Melo and Hebert 1994). As the combinatorial analyses suggested that insufficient loci were examined to identify all of the taxa present in samples, the detection of such amalgams was not unexpected. The joint morphological and genetic analyses indicated the presence of 10 *Bosmina* taxa, of which six had been recognized by prior taxonomic work (Deevey and Deevey 1971; Paggi 1979; Lieder 1983b). Analysis of the relationship between species detection and the number of sites analyzed suggested that sufficient habitats were sampled to encounter all of the species present in the sampling area. However, as most of the samples arose from a small part of the continent, it is very likely that additional taxa await discovery in other areas of North America. When coupled with the results of earlier studies on genetic diversity in bosminids (Manning et al. 1978; Brock 1980), the results emphasize the need for a reevaluation of both past studies of "intraspecific" variation in morphology among bosminids (Kerfoot 1975a, 1975b, 1980), and the presumption of cosmopolitanism, as it now seems likely that this work has involved the comparison of different species or even members of different subgenera. For example, the species *B.* (*B.*) *longirostris* is reported to be distributed worldwide (Lieder 1983b), yet we detected this species in only one habitat of those surveyed. Clearly, the restricted distribution of

TABLE 4. Mean genetic characteristics and mean sample size per locus for North American bosminids at the subgeneric and species levels. N = number of populations collected for that taxon, n = number of individuals analyzed, A = range of mean number of alleles per locus, P = percentage polymorphic loci (0.95 criterion), H_0 = observed heterozygosity, HW = total number of tests for Hardy–Weinberg equilibrium, D = number of tests showing significant heterozygote deficiencies from Hardy–Weinberg expectations, E = number of tests showing significant heterozygote excesses from Hardy–Weinberg expectations.

Subgenus	Species	M	n	A	P	H_0	HW	D	E
Bosmina s. s.		1	81	1.6	50.0	0.42	4	—	3
Sinobosmina				1.31	19.8	0.08			
	A	11	481	1.34	21.6	0.08	23	2	4
	B	7	242	1.27	17.9	0.07	8	2	—
Neobosmina				1.07	5.6	0.06			
	A	3	132	1.03	4.2	0.14	1		
	B	2	110	1.0	0.0	0.00	—	—	—
	C	2	33	1.2	12.5	0.04	2	—	—
Eubosmina				1.23	13.0	0.06			
	A	14	339	1.14	11.6	0.02	11	4	1
	B	2	82	1.1	12.5	0.12	2	—	2
	C	4	170	1.28	3.1	0.02	1	—	—
	coregoni	26	2767	1.28	24.8	0.08	34	11	4
All samples		72	4437		15.8 (12.0)[a]	0.10 (0.06)[a]	86	19	14

[a]Excluding *Bosmina s. s.*

B. (*B.*) *longirostris*, as well as the morphological and genetic similarity between this species and members of the subgenus *Sinobosmina*, suggests that the long-held assumption of cosmopolitanism in the species is incorrect.

If the extent of genetic divergence is a reliable correlate of taxonomic separation, then one would expect members of individual subgenera to show both genetic cohesion and clear genetic divergence from members of other subgenera. In fact, this was only the case for members of the subgenus *Eubosmina*. Assuming an allozyme divergence rate of 1 D = 5.6 MYr (Nei 1987), the genetic distance between this subgenus and the remaining bosminids (D = 1.2) suggests their divergence approximately 7 million yr ago. The subgenera *Neobosmina* and *Sinobosmina* were both suggested by allozymic data to be polyphyletic, a result that casts doubt on both the validity of the subgeneric classifications and the strength of the diagnostic characters. However, the failure of allozyme data to support the monophyly of subgenera aside from *Eubosmina* may be a consequence of the small number of allozyme loci employed in this analysis.

Although Lieder (1983a) suggested that hybridization was common in European bosminids, no firm evidence for its occurrence was detected in this study. Species belonging to different subgenera co-occurred in 12 of 45 lakes, but hybrids between them were never detected, although allozyme markers would have permitted their recognition. Similarly, hybrids were not detected when members of the same subgenus co-occurred, as, for example, when *Sinobosmina* sp. *A.* and sp. *B.* co-occurred in three lakes. Two of the taxa detected in this study may have arisen as a result of interspecific hybridization, but if so, one of the parental species awaits detection. The sole *Bosmina s. s.* population might have arisen from hybridization between *Sinobosmina* sp. *A* and a species showing the appropriate allelic substitution at AAT. Similarly, *Eubosmina* sp. *B* might have arisen from hybridization between *E. coregoni* and an unknown member of this subgenus with a substitution at MDH.

Populations of bosminids were, on average, polymorphic at 15.8% of their loci, a value approximately half that reported for other Crustacea (Nevo et al. 1983) but similar to that seen in other freshwater zooplankton (Hebert 1980; Hann and Hebert 1986; Boileau and Hebert 1988; Taylor and Hebert 1992). Individual heterozygosities in bosminids averaged 10%, a value comparable with that reported for many other invertebrates (Nevo et al. 1983). Significant Hardy–Weinberg deviations were detected at 38% of polymorphic loci. In some species, deviations were usually due to heterozygote deficiencies, as noted earlier by Brock (1980), and may have resulted either from inbreeding or from the presence of unrecognized species (Hebert 1980; Hann and Hebert 1986). Heterozygote excesses were, however, noted in populations of both *Bosmina s. s.* and *Eubosmina* sp. *B.* Heterozygote excesses in other cladocerans have been associated with the transition to obligate parthenogenesis (Crease et al. 1989), to extended periods of parthenogenesis in cyclic asexuals (Manning et al. 1978; Hebert 1980), and to interspecific hybridization (Hebert 1985).

We have demonstrated that species diversity in North American members of the Bosminidae is considerably higher than previously recognized. This taxonomic reevaluation has secondary impacts: phenotypic diversity is probably more constrained in single species than previously believed, and species distributions are also more limited. The latter result suggests that dispersal abilities of bosminids may be sufficiently limited to permit allopatric speciation. However, the discovery of gene pool divergence between pond and lake populations of several species also suggests that disruptive selection linked to habitat diversification (Maynard Smith 1966; Tauber and Tauber 1977) may play an important role in the speciation of these organisms.

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Oceanographic Basis of a DMS-Related Atlantic Cod (*Gadus morhua*) Fishery Problem: Blackberry Feed

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Atlantic cod (*Gadus morhua*) caught along the Labrador and Newfoundland coasts and in the Strait of Belle Isle often smell strongly of sulfur, a problem locally called "blackberry feed". Early studies have shown that the sulfurous-smelling cod had fed almost exclusively on the pteropod *Limacina helicina* and that dimethylsufide (DMS) was responsible for the odor. In August 1991, we conducted a cruise in the northeastern Gulf of St. Lawrence and in the Strait of Belle Isle in order to confirm the algal origin of dimethylsulfoniopropionate (DMSP, the precursor of DMS) and to determine the factors responsible for its transfer in the food web. A significant correlation was found between the distribution of DMSP and the abundance of microflagellates, dinoflagellates, cryptomonads, and prymnesiophytes (mostly *Chrysochromulina* spp.). The concentration of DMSP in *L. helicina* and in particles were also linearly related, suggesting rapid accumulation of DMSP in pteropod. The weight-specific DMSP content of *L. helicina* was always high (1–21 mg DMSP/g) and apparently sufficient to create blackberry feed symptoms in cod. Results from bioassay experiments demontrated that *L. helicina* may significantly influence the DMSP–DMS dynamics in subarctic waters.

Les morues franches (*Gadus morhua*) capturées le long des côtes du Labrador et de Terre-Neuve et dans le détroit de Belle Isle sont régulièrement caractérisées par une forte odeur de soufre, un problème désigné localement « blackberry feed ». Les résultats de premiers travaux ont démontré que ces morues s'étaient alimentées presqu'exclusivement du ptéropode *Limacina helicina* et que le sulfure de diméthyle (DMS) était responsable de l'odeur. En août 1991, nous avons effectué une mission dans la partie nord-est du golfe du Saint-Laurent et dans le détroit de Belle Isle dans les buts de confirmer l'origine algale du diméthylsulfoniopropionate (DMSP), le précurseur du DMS, et de déterminer les facteurs responsables de son transfert dans la chaîne trophique. La répartition du DMSP était significativement corrélée avec l'abondance des microflagellés, dinoflagellés, cryptomonades et des prymnesiophytes (principalement *Chrysochromulina* spp.). Le contenu en DMSP de *L. helicina* était corrélé avec celui des particules, suggérant une accumulation rapide du DMSP dans les ptéropodes. Les concentrations en DMSP des ptéropodes normalisées par unité de poids étaient toujours élevées (1–21 mg DMSP/g) et apparemment suffisantes pour générer les symptômes du « blackberry feed ». Les résultats de bio-essais ont démontré que *L. helicina* pouvait influencer significativement la dynamique du DMSP–DMS dans les eaux subarctiques.

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In the marine environment, dimethylsufide (DMS) is produced by the cleavage of dimethylsulfoniopropionate (DMSP)[1], a compound involved in osmoregulation in algae (Ackman et al. 1966b). The conversion of DMSP into DMS is enhanced by cell lysis, bacterial activity, and zooplankton grazing (Dacey and Wakeham 1986; Nguyen et al. 1988; Belviso et al. 1990; Kiene and Service 1991). A percentage of the DMS produced diffuses to the atmosphere where, following its photooxidation to sulfur dioxide, it contributes to particulate sulfate and concentrations of cloud condensation nuclei. DMS is thus suspected to influence the earth's albedo and regional climate (see review by Malin

[1]Analogous to dimethyl-β-propiothetin (DMPT).

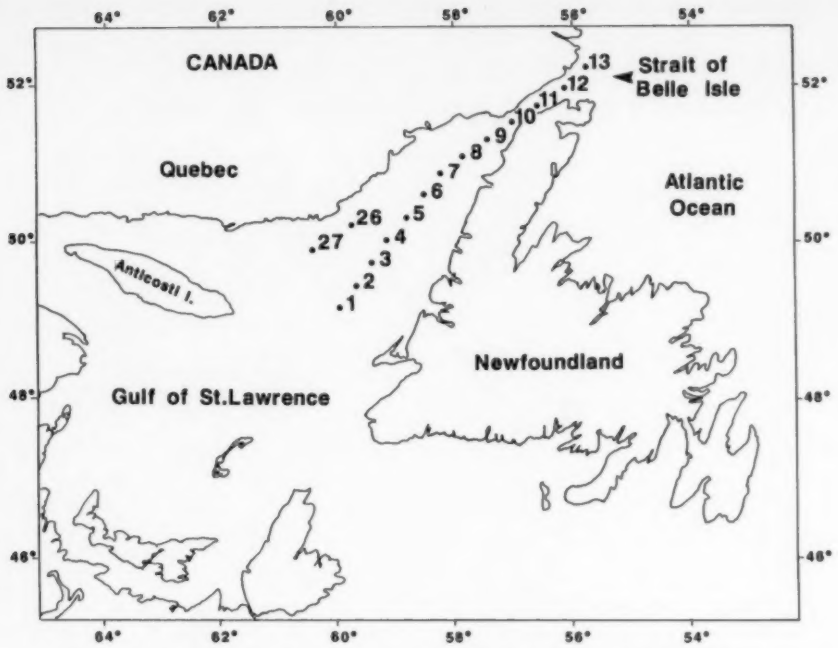

FIG. 1. Northeastern Gulf of St. Lawrence and location of sampling stations.

et al. 1992). Although less publicized, DMS also affects fish quality by imparting an undesirable sulfur smell to various sea products (Motohiro 1962; Sipos and Ackman 1964; Lippa 1965; Ackman et al. 1967, 1972; Botta et al. 1985; Iida and Tokunaga 1986; Iida et al. 1986).

On the Canadian east coast, DMS has been detected regularly in Atlantic cod (*Gadus morhua*) caught offshore of Labrador and Newfoundland (Sipos and Ackman 1964). Locally called "blackberry feed," the problem lasts 2–3 wk in July–August and often results in important financial loss to fishermen. Although DMS is assumed to be of algal origin (Sipos and Ackman 1964), it is unknown which species are responsible for its production offshore of Labrador and Newfoundland. DMSP production within phytoplankton is highly species specific. Dinoflagellates and prymnesiophytes (particularly the coccolithophores) contain high levels of DMSP, while diatoms, and other classes, produce very little (Keller et al. 1989a). The phytoplankton composition of the northeastern part of the Gulf and of the Labrador Current is poorly known. Early observations showed that the sulfur-smelling cod fed almost exclusively on *Limacina helicina*, a common suspension-feeding pteropod in arctic and subarctic waters (Kerswill 1940). This pteropod has been implicated previously in the transmission of DMS from algae to fish. In the North Pacific, Motohiro (1962) isolated DMSP from *L. helicina*, found in the viscera of sulfurous-smelling salmon. Pteropods may be particularly efficient in the accumulation of DMSP.

The objectives of this study were to determine the algal origin of DMSP in the Gulf of St. Lawrence and Labrador Current and the role of the pteropod *L. helicina* in the transfer of DMSP to cod. In addition, bioassay experiments were conducted to determine the influence of pteropods on DMSP–DMS dynamics in these temperate–subarctic waters.

Materials and Methods

Water and Zooplankton Sampling

Between 17 and 29 July 1991, we sampled 13 stations located along a transect covering the central part of the Gulf of St. Lawrence, the Strait of Belle Isle, and the Labrador Current (Fig. 1). Data from four additional stations located in the Strait (perpendicular to the transect, both sides of stations 10) and two stations along the north shore of the Gulf (stations 26 and 27) were used in determining the relationship between DMSP concentration in the water and the weight-specific DMSP content of the pteropods. At each station, vertical profiling of temperature, salinity, and fluorescence was conducted using a rosette equipped with a Guildline CTD and a Seatech fluorometer. Water samples were collected at five depths (surface, middle of the surface mixed layer, fluorescence maximum, 100 m, and near the bottom) with 5-L GO-FLO bottles. Immediately after the rosette cast, zooplankton were sampled with two 330-μm-mesh nets, mounted on a 1-m^2 metal frame equipped with a system which allows independent opening of each net at a given depth. The nets were fitted with internal flowmeters and a 3-L rigid codend with 80-μm-mesh apertures. The trawl was first sent to the bottom with the nets closed and then towed obliquely toward the surface with the ship travelling at approximately 3 kn. Zooplankton were collected in two strata: from the bottom to the lower part of the mixed layer (exclusive of the subsurface chlorophyll maxima, SCM) and from the SCM to the surface.

Chlorophyll, Nutrients, and Phytoplankton Analysis

From each water sample, two 250-mL subsamples were filtered on Whatman GF/F filters, extracted for 24 h in 90%

acetone, and analyzed for chlorophyll *a*, using the fluorometric method of Yentsch and Menzel (1963) as modified by Holm-Hansen et al. (1965). An additional 50-mL subsample was filtered through a GF/F filter and the filtrate collected for the measurement of nutrients (ammonium-NH_4, phosphate-PO_4, nitrate-NO_3, and nitrite-NO_2) onboard using a Technicon AutoAnalyser (Parsons et al. 1984). A subsample of the filtrate was frozen for analysis of silicate (Parsons et al. 1984) and urea (diacetyl monoxime thiosemicarbazide technique described by Aminot and Kerouel 1982 and Price and Harrison 1987) upon return to shore. Thawed nutrient samples were kept at 4°C for 24 h before silicate determination. Two 250-mL subsamples were fixed with either 5 mL of formaldehyde (4%) or acidic Lugol's fixative for later identification and counting of coccolithophores and diatoms/flagellates, respectively, using the Utermöhl technique (Lund et al. 1958).

Determination of DMSP in Particulate and Dissolved Matter

Concentrations of DMSP were determined at the surface and SCM depths at all stations and at all depths at stations 8, 10, and 13. One millilitre of 5 M NaOH was placed in 25-mL vials which were then filled with sample and immediately sealed with a Teflon-faced serum cap. These samples were held in the dark at room temperature until analysis. In the laboratory, DMSP (as DMS) was measured on a Varian 3300 gas chromatograph equipped with flame photometric detection and a Chromosil 330 Teflon column, as described in Keller (1991). Samples of DMS analysis (1–10 mL) were sparged with N_2 gas (30 mL/min) and cryotrapped in a Teflon loop submerged in liquid nitrogen. The Teflon loop was subsequently heated (approximately 80°C), releasing the collected sample onto the column. Using the specific reaction which cleaves DMSP into equimolar amounts of DMS upon alkali treatment (Challenger and Simpson 1948), the measured quantity of DMS in unfiltered samples represented the total amounts of DMS and DMSP ($DMSP_t$ = particulate DMSP ($DMSP_p$) + dissolved DMSP ($DMSP_d$) + free DMS), while the quantity of DMS measured in samples filtered through Whatman GF/F filters represented the amount of dissolved DMSP and free DMS ($DMSP_d$ + DMS). Particulate DMSP ($DMSP_p$) was computed as the difference $DMSP_t - DMSP_d$. Standards, prepared from known quantities of DMSP, were analyzed in similar fashion.

Determination of DMSP in *Limacina helicina*

Upon retrieval, the net was thoroughly rinsed, the pteropods *L. helicina* were immediately sorted out, and the rest of the zooplankton sample was preserved in 4% formalin. For DMSP measurements, five specimens of *L. helicina* were measured (diameter) and placed in 25-mL vials containing 1 mL of 5 M NaOH and then filled with demineralized water (containing no DMS or DMSP). $DMSP_t$ was measured in these samples as described above. To standardize the DMSP content of *L. helicina* per unit weight, 25 specimens of the pteropod were measured (diameter) and kept frozen for later determination of dry weight.

Bioassay Experiments

Three bioassays were conducted on board with freshly caught *L. helicina* to examine their influence on DMSP and DMS dynamics (via ingestion and excretion). In bioassays 1 and 2, pteropods captured in the upper 20 m of the Labrador Current (station 13) were incubated for 12 h in two 2-L reservoirs (five pteropods per reservoir) filled with prescreened (230-μm Nytex) seawater collected at the same station. In bioassay 2, five additional pteropods were incubated in a third reservoir filled with filtered seawater (GF/F) to remove the potential influence of microzooplankton <230 μm on DMSP/DMS dynamics. Bioassay 3 was conducted as bioassay 2, but the pteropods were incubated with prescreened seawater collected at station 10 in the Strait of Belle Isle where the phytoplankton biomass was higher than in the Labrador Current. All incubations were done in the dark (to prevent phytoplankton growth) and at 4°C, the mean temperature measured at the station where the plank-

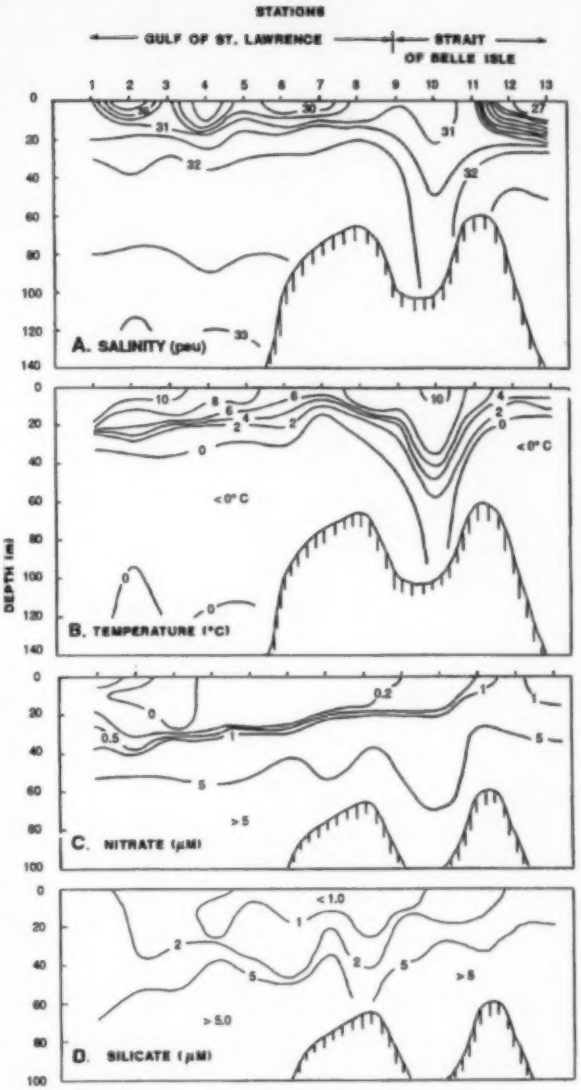
STATIONS
GULF OF ST. LAWRENCE
STRAIT OF BELLE ISLE
1 2 3 4 5 6 7 8 9 10 11 12 13
DEPTH (m)
A. SALINITY (psu)
B. TEMPERATURE (°C)
C. NITRATE (μM)
D. SILICATE (μM)

FIG. 2. Cross-sections of (A) salinity, (B) temperature, (C) nitrate, and (D) silicate along the south–north transect.

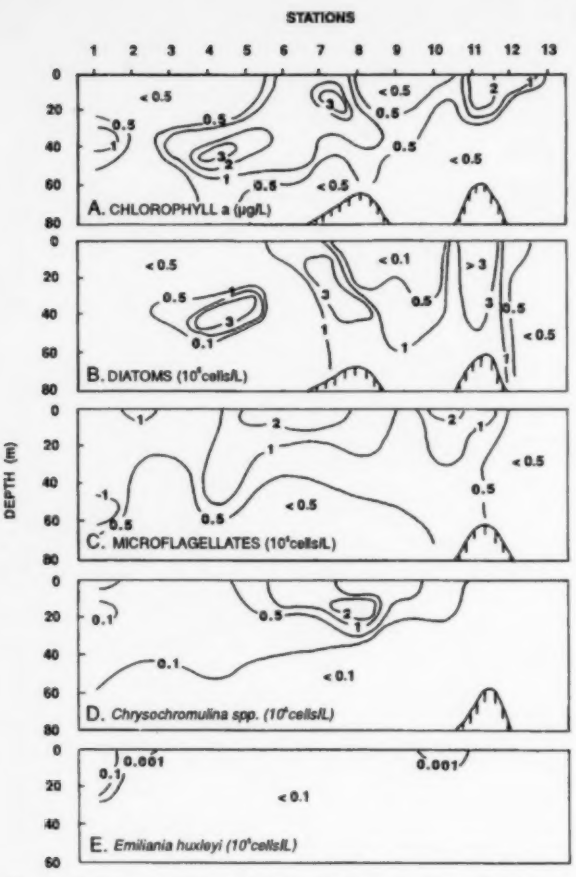
STATIONS
1 2 3 4 5 6 7 8 9 10 11 12 13
DEPTH (m)
A. CHLOROPHYLL a (µg/L)
B. DIATOMS (10⁴cells/L)
C. MICROFLAGELLATES (10⁴cells/L)
D. Chrysochromulina spp. (10⁴cells/L)
E. Emiliania huxleyi (10⁴cells/L)

FIG. 3. Cross-sections of (A) chlorophyll *a* and of the abundance of (B) diatoms, (C) microflagellates, (D) *Chrysochromulina* spp., and (E) *E. huxleyi* along the south–north transect.

ton were collected. Controls consisted of two additional reservoirs filled with seawater and incubated similarly, without pteropods. The following variables were measured in duplicate in each reservoir at the beginning (t_0) and at the end (t_{12}) of the 12-h incubation: chlorophyll *a* (as an index of feeding), phaeopigments (as an index of excretion), and $DMSP_p$ and $DMSP_d$ + free DMS.

Results

Hydrography and Nutrient Concentrations

Except for stations 12 and 13, which were located in the Labrador Current, there was little variation in surface salinity across the transect (approximately 30 psu) (Fig. 2A). Salinity decreased sharply between stations 11 (31 psu) and 12 (27.9 psu) when the ship entered into the Labrador Current. Surface temperatures were highest in the central part of the Gulf (11.6°C; stations 1–6), decreased to 6°C at station 7, as the ship moved toward the shallower northeastern part of the Gulf, increased to 10°C in the Strait (stations 8–11), and decreased drastically to 4.7°C in the Labrador Current (stations 12 and 13) (Fig. 2B). The surface mixed layer depth varied from 20 m to 10 m between

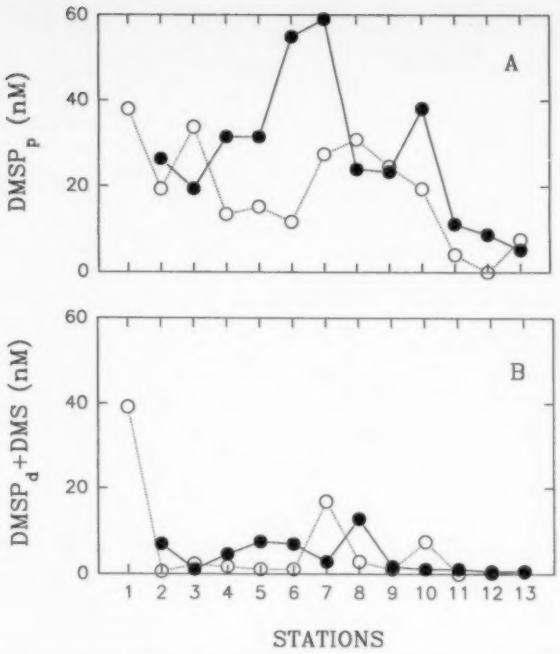

FIG. 4. (A) $DMSP_p$ and (B) $DMSP_d$ + free DMS concentrations near the surface (solid line and symbols) and in the subsurface chlorophyll maximum (dotted line, open symbols) along the south–north transect.

stations 1 and 8 in the Gulf, increased to 40 m in the Strait, and decreased again to <10 m in the Labrador Current.

Nitrate and silicate concentrations in the upper mixed layer were low (<0.3 and <2.0 µM, respectively) at most stations along the transect, as expected in the Gulf during midsummer (Fig. 2C and 2D). Nitrate and silicate concentrations increased to 1.7 and 4 µM , respectively, at stations 12 and 13 in the Labrador Current. Phosphate and regenerated sources of nitrogen (not shown) were relatively high in the mixed layer at all stations (PO_4 = 0.35 µM; NH_4 = 0.2–1.0 µM; urea = 0.5–2.5 µM).

Phytoplankton Composition and Biomass Distribution

Changes in phytoplankton biomass (chlorophyll *a*) and composition were observed across the transect. Maximum chlorophyll *a* was recorded at depth (25–40 m) in the Gulf, near the surface in the northeastern part of the Gulf, and at station 11 in the Strait (Fig. 3A). Chlorophyll *a* concentrations were low (<0.3 µg/L) in the Gulf upper mixed layer and in the Labrador Current. Diatoms, dominated by *Chaetoceros socialis*, were concentrated in the Strait and formed a subsurface maximum in the Gulf (Fig. 3B). Microflagellates, the second most abundant algal group, were concentrated in the upper 20 m at stations 5–11 (Fig. 3C). *Chrysochromulina* spp. was the most abundant group of prymnesiophytes, reaching 2×10^6 cells/L at stations 6–9 in the Gulf (Fig. 3D). *Emiliania huxleyi*, the second most abundant prymnesiophytes, was found only in the upper mixed layer in the Gulf and in the Strait where surface temperatures exceeded 9°C (Fig. 3E). The concentration of coccoliths was relatively low (max. = 8×10^6 coccoliths/L)

TABLE 1. Pearson's coefficient of linear correlation (r^2) between $DMSP_p$, $DMSP_d$ + DMS, chlorophyll *a*, and the abundance of the major phytoplankton groups (*$P \leq 0.01$; $n = 33$).

	$DMSP_p$	$DMSP_d$ + DMS
Chlorophyll *a*	0.03	0.08
Diatoms	0.01	0.00
Microflagellates	0.48*	0.03
Dinoflagellates	0.45*	0.20
Chrysophytes	0.16	0.00
Cryptomonads	0.41*	0.24*
Prymnesiophytes	0.42*	0.01
Chrysochromulina spp.	0.43*	0.10
Emiliania huxleyi	0.03	0.00

and the coccolith/cell ratio never exceeded 60. Dinoflagellates and cryptomonads were found in low abundance ($<0.2 \times 10^6$ cells/L) along the transect (not shown).

Particulate and Dissolved DMSP

Particulate DMSP concentrations varied from <0.3 nM (detection limit) to 59 nM, while dissolved DMSP and DMS (single value) concentrations ranged from nondetectable to 39 nM (Fig. 4). $DMSP_p$ concentrations were positively correlated with the abundance of microflagellates, dinoflagellates, cryptomonads, and *Chrysochromulina* spp., while no relationship was found between the distribution of $DMSP_p$ and chlorophyll *a* (Table 1). The distribution of $DMSP_d$ + DMS was not correlated with the distribution of $DMSP_p$, but a weak correlation was found between $DMSP_d$ + DMS and the abundance of cryptomonads (Table 1).

Limacina helicina Distribution and DMSP Content

The pteropod *L. helicina* was present at most stations along the transect but was only abundant in the Labrador Current (Fig. 5A). While the mean diameter of the pteropod varied from 0.7 to 4.6 mm, individuals with diameters >2 mm, such as those normally found in cod, were captured in very low abundance in the Gulf and in higher abundance in Labrador Current waters (Fig. 5B). Larger animals contained generally more DMSP (up to 14 μg DMSP/individual) than smaller ones (Fig. 5C). The relationship between dry weight and diameter of *L. helicina* is given by

$$\text{Dry weight (mg)} = e^{-2.034 + 0.687\,[\text{diameter (mm)}]}.$$

When normalized to dry weight, bigger individuals captured in the Labrador Current had the lowest weight-specific DMSP content (Fig. 5D).

The mean diameter of *L. helicina* and its DMSP content were correlated ($r^2 = 0.65$; $P < 0.01$; $n = 14$; Fig. 6A) as were the weight-specific DMSP content of *L. helicina* and the concentration of ambient $DMSP_p$ in the upper mixed layer ($r^2 = 0.72$; $P < 0.01$; $n = 14$; Fig. 6B).

Bioassay Experiments

Replication was good between paired bioassay treatments, so data were combined (error bars in Fig. 7). Bioassays 1 and 2 started with the low ambient concentrations of chlorophyll *a*, phaeopigment, and DMSP of the Labrador Current (Fig. 7A and 7B); after 12 h, no significant changes in these variables were recorded in the controls. Although the pteropods swam normally during incubations, the absence

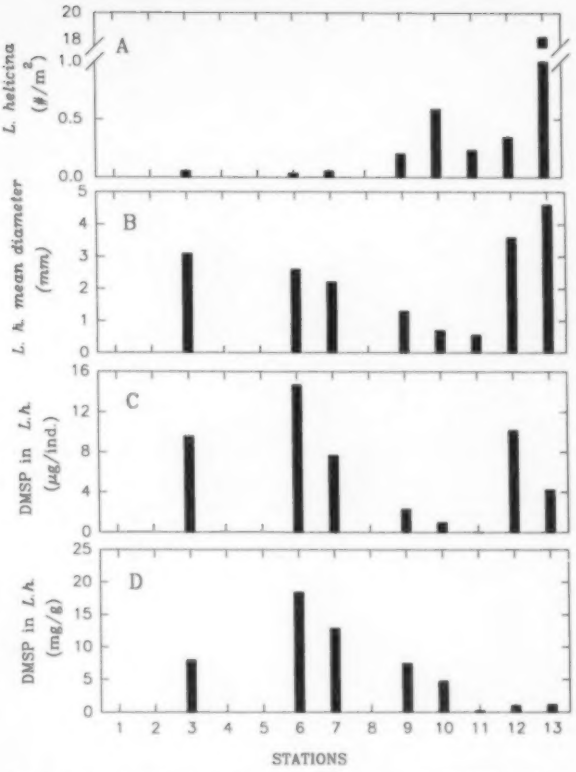

FIG. 5. (A) Abundance, (B) mean diameter, (C) DMSP content per individual, and (D) weight-specific DMSP content of *L. helicina* along the south–north transect.

of significant change in chlorophyll *a* after 12 h suggests that they did not feed. Excretion by the pteropods resulted in a three- and ninefold increase in phaeopigment concentration, a 20- and 21-fold increase in $DMSP_p$ concentration, and a 25- and 24-fold increase in $DMSP_d$ + free DMS concentration in bioassays 1 and 2, respectively (Fig. 7A and 7B). Overall, the presence of the pteropods resulted in a 21- and 22-fold increase in total DMSP and free DMS in the water in bioassays 1 and 2, respectively. In bioassay 2, excretion of DMSP and DMS was similar in pteropods incubated in filtered and unfiltered seawater (*t*-test; $P \geq 0.05$; Fig. 7C), indicating that microzooplankton (<230 μm) were not important in the observed production of DMSP and DMS. Although chlorophyll *a* concentration in bioassay 3 was 13 times higher than in bioassay 1 or 2, feeding activity of pteropods was not enhanced, but excretion of DMSP and DMS did increase similar to bioassays 1 and 2 (Fig. 7D). On average, the excretion of DMSP and DMS by a single pteropod was 2126 ng/individual, a value corresponding to 56% of initial internal levels (Table 2).

Discussion

Algal Origin of DMSP in the Gulf of St. Lawrence and Labrador Current

Particulate DMSP concentrations were maximal in the Gulf and minimal in the Labrador Current. The mean value in the Gulf (28 nM) was intermediate to previously reported

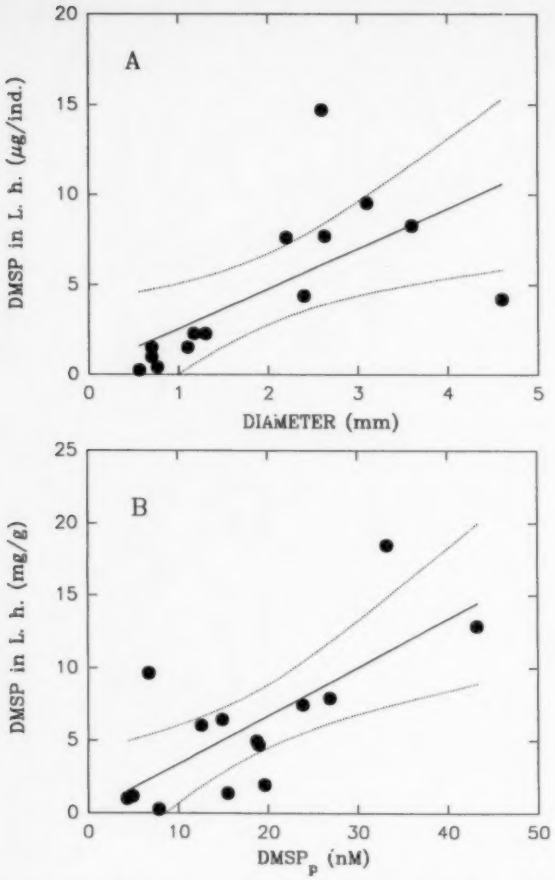

FIG. 6. Relationships between (A) the DMSP content of *L. helicina* and its diameter and (B) the weight-specific DMSP content of *L. helicina* and the ambient DMSP$_p$ concentrations.

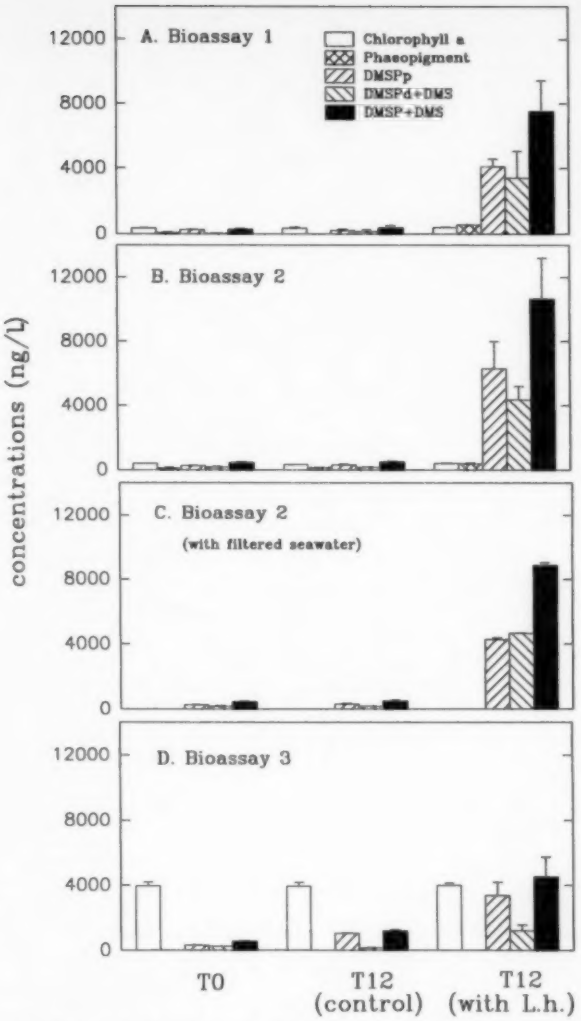

FIG. 7. Results from the 12-h bioassays conducted with *L. helicina* from the Labrador Current incubated in (A) unfiltered seawater collected at station 13, (B) unfiltered and (C) filtered seawater collected at station 21, and (D) unfiltered seawater collected at station 10.

amounts for summer values for the open Atlantic (4.6 nM; Burgermeister et al. 1990) and around mainland Britain (89 nM; Turner et al. 1988), but maximum values measured in the Gulf were considerably less than those reported previously in coastal waters: 286 nM (Turner et al. 1988) and 160 nM (Iverson et al. 1989). In the Labrador Current, the mean DMSP$_p$ concentration of 6 nM was low but similar to that reported by Burgermeister et al. (1990) for the mid-Atlantic.

The lack of correlation between the distribution of DMSP and total chlorophyll in the Gulf is not unexpected because most of the chlorophyll variability can be attributed to diatoms which produce little DMSP (Keller et al. 1989a). Along the transect, microflagellates, dinoflagellates, prymnesiophytes, and cryptomonads were primarily responsible for variations in DMSP. Species belonging to the classes Dinophyceae and Prymnesiophyceae have been shown to produce DMSP (Keller et al. 1989a, 1989b). The microflagellates may have been dominated by chromophytes, belonging to the classes of Prymnesiophyceae and Chrysophyceae, both DMSP producers. The correlation with cryptomonads is somewhat enigmatic.

Results from the correlation analysis suggest that the prymnesiophytes *Chrysochromulina* spp. are important DMSP producers in the Gulf of St. Lawrence. *Chrysochromulina* was also correlated previously with high DMS levels in the western English Channel (Holligan et al. 1987), but not in coastal Scandinavian waters (Leck et al. 1990). *Chrysochromulina* spp. were particularly abundant in the northeastern part of the Gulf, an area characterized by relatively low surface temperatures. The *T/S* and nutrient characteristics of the surface waters in this area suggest that the water was upwelled from depth (approximately 10–20 m) in the Gulf. The growth of *Chrysochromulina* may thus have been favored by a previous wind-driven upwelling event, as is frequently observed in this area (Rose and Leggett 1988).

The coccolithopore *E. huxleyi*, a significant DMSP producer (Keller et al. 1989a), was also present in moderate

TABLE 2. $DMSP_p$, $DMSP_d$ + DMS, and total excretion rates of *L. helicina* in the 12-h bioassays, internal total concentrations measured in *L. helicina* at the end of the bioassays, and percentage of the initial DMSP + DMS content of *L. helicina* excreted during the bioassays (1 SD in parentheses).

Bioassay	Variable	Excretion rate ($ng \cdot ind.^{-1} \cdot 12\ h^{-1}$)	*L. h.* residual content (ng/ind.)	Initial content excreted in 12h[a] (%)
1	$DMSP_p$	1560 (201)	—	—
	$DMSP_d$ + DMS	1303 (699)	—	—
	Total	2863 (826)	2602 (163)	52
2 (unfiltered)	$DMSP_p$	2392 (670)	—	—
	$DMSP_d$ + DMS	1671 (338)	—	—
	Total	4063 (1004)	2182 (3)	65
2 (filtered)	$DMSP_p$	1577	—	—
	$DMSP_d$ + DMS	1792	—	—
	Total	3369	1948	63
3	$DMSP_p$	928 (318)	—	—
	$DMSP_d$ + DMS	414 (138)	—	—
	Total	1342 (454)	1772 (69)	43

[a]Initial internal content in DMSP + DMS was calculated as the sum of the excretion rate and the residual content measured in *L. helicina* at the end of the incubation.

concentrations (<1.5×10^5 cells/L) at stations exhibiting surface water temperatures higher than 9°C (stations 1–3 in the central part of the Gulf and station 10 in the Strait). Correlation between DMSP and *E. huxleyi* is poor probably because of its rare occurrence in this data set. *Emiliania huxleyi* growth may have been temperature limited in the Gulf, or it may require higher solar irradiance or enhanced vertical stability. Blooms of this organism have been reported from many locations and are easily identified by satellite imagery because they shed their calcium carbonate plates, the coccoliths, into the surrounding water as they grow. In concentration, these coccoliths produce a strong backscattering signal, which is easily detected from space. The release of the coccoliths occurs in older stages of a bloom and can reach concentrations of 10^9/L (Balch et al. 1991). The concentration of coccoliths in the Gulf was relatively low (max. = 8×10^6/L), and the coccolith/cell ratio never exceeded 60. This low ratio suggests that the population was in an early phase of development and probably explains why it remained undetected in satellite images (C.A. Brown and J.A. Yoder, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA, personal communication). Satellite imagery from previous years suggests that *E. huxleyi* has formed large blooms in the Gulf in July and August (Brown and Yoder 1994). Matrai and Keller (1993) examined DMS and DMSP dynamics in a bloom of *E. huxleyi* in the Gulf of Maine, where cell concentrations reached 3×10^6/L and coccolith concentrations were 1–2 orders of magnitude higher. Concentrations of up to 375 nM DMSP were associated with the concentrations of coccolithophores, and partitioning between dissolved and particulate DMSP changed in relation to bloom age. In older parts of the bloom, where the coccolith/cell ratio was highest, a substantial percentage of the DMSP was in the dissolved form and DMS was highest. *Emiliania huxleyi* may thus represent another potentially important DMSP producer in the Gulf of St. Lawrence.

Dissolved DMSP and free DMS concentrations were always lower than particulate DMSP concentrations (approximately factor 10), except at station 1 where levels were equivalent. The lowest $DMSP_d$ + free DMS concentrations were found in Labrador Current waters. Although spatially similar, distributions of the different DMSP fractions were not correlated, a situation which is not uncommon because the degradation of cellular DMSP into dissolved DMSP and DMS is affected by the physiological state of phytoplankton, grazing by zooplankton, and bacterial activity (Dacey and Wakeham 1986; Nguyen et al. 1988; Belviso et al. 1990; Kiene and Service 1991; Matrai and Keller 1993). Decoupling can thus be expected between these pools.

Transfer of DMSP to Cod from *L. helicina* in the Labrador Current

Our results confirm that *L. helicina* found in the Strait of Belle Isle in summer originates in the Labrador Current. Concentrations of this pteropod in the cold surface waters of the Labrador Current were 30 times higher than in the Strait and in the Gulf. The 1991 summer was exceptionally cold, with very high densities of drifting ice in the Labrador Current and the Strait of Belle Isle. These conditions may have negatively affected the reproduction and development of *L. helicina*. The mean diameter varied by a factor of 6 along the transect, with smaller individuals found in the Strait. The size of *L. helicina* depends on both its developmental stage and nutritional status (C.M. Lallie, Department of Zoology, University of British Columbia, Vancouver, BC V6T 1W5, Canada, personal communication). The small size of individuals captured in the Strait may thus represent either immature or starved individuals, although high chlorophyll in the Strait indicates abundant food supply. However, these high values can largely be attributed to diatoms which may be a poor food choice for *L. helicina*. Gilmer and Harbison (1991) described the diet of adult *L. helicina* as consisting mainly of microzooplankton and found additionally that diatoms, while abundant in the food supply, may pass intact through the guts of these pteropods. Thus, the possibility exists that *L. helicina* accumulates DMSP not directly from a phytoplankton source, but indirectly by feeding upon organisms which in turn have consumed the algae. Although the concentrations of *L. helicina* in the Strait and Labrador Current were probably too low in 1991 to initiate the blackberry feed symptoms in cod

TABLE 3. Mean $DMSP_p$ concentrations in the upper mixed layer, DMSP content of *L. helicina*, volume of water needed to be cleared by *L. helicina* to obtain its DMSP content, and minimum number of pteropods that a cod must theoretically ingest per day to assimilate 10 mg of DMSP, the minimum amount required to develop the blackberry symptoms.

Station	Mean $DMSP_p$ in water (ng/L)	DMSP in *L. helicina* (ng/ind.)	Equivalent volume of water (L)	Minimum cod diet (no. of pteropods)
3	4589	9536	2.1	1048
6	5661	14705	2.6	680
7	7361	7655	1.0	1306
9	4063	2323	0.6	4304
10	3230	1001	0.3	9990
11	1326	1544	1.2	6476
12	731	8281	11.3	1207
13	833	4227	5.1	2365

(maximum of 18 individuals/m^2), we are able to speculate about the factors responsible for the accumulation of DMSP in the pteropod.

The turnover rate of DMSP and DMS in *L. helicina* appears to be very fast. Each whole, larger pteropod contains approximately 6159 ng of DMSP (Fig. 5C; Table 3), and 43–65% of this amount can be excreted in less than 12 h (Table 2). When compared with the mean $DMSP_p$ concentrations found at the different stations, each pteropod must have cleared on average 3 L of seawater with 100% efficiency to reach its DMSP quota (Table 3). The feeding behavior of this organism involves the production of an external mucus web to collect particles (Gilmer and Harbison 1986). To our knowledge, this organism has never been successfully maintained in captivity and daily clearance rates are not known.

The excretion rates of *L. helicina* determined during our bioassays suggest a rough estimate of clearance rate. Assuming that the DMSP concentrations measured during the 12-h experiments represent a good estimate of the gut content of the organism, our measurements indicate that the pteropods captured at station 13 had recently (during a 12-h period) cleared 3 L of seawater, giving a daily clearance rate as high as 6 L. This estimate of daily clearance rate may be too high due to potential effect of stress on excretion. Alternatively, if *L. helicina* feeds selectively on microzooplankton, as indicated by Gilmer and Harbison (1991), which presumably also accumulate DMSP, the clearance rates might be considrably less. Our sample sizes for DMSP analysis, while suitable for phytoplankton, would not sample microzooplankton quantitatively or qualitatively. The high levels of regenerated nitrogen suggest an active microbial web, of which microzooplankton might be an integral part. *Calanus pacificus*, a suspension-feeding copepod of comparable size to *L. helicina*, has a maximum clearance rate of 0.5 L/d (Runge 1980).

The significant relationship observed between the weight-specific DMSP content of pteropod and the DMSP content of their food (ambient $DMSP_p$ concentrations) supports the conclusion that DMSP turnover rate is rapid in *L. helicina*. If accumulation occurred over time, the two would be uncoupled. The weight-specific DMSP content of the animals measured in this study (1–21 mg/g) was higher than the values previously measured in pteropods from Baffin Bay (0.2–0.5 mg/g; Ackman et al. 1966a) and in the Pacific (0.02 mg/g; Motohiro 1962). The significant relationship between the weight-specific DMSP content of the pteropod and DMSP in water samples in our study suggests that differences between reported values may be due to variations in DMSP concentrations in prey. There are no published reports, to our knowledge, of endogenous production of DMSP by any heterotrophic organism. The possibility does exist, but if it were significant, there should be no relationship between predator and prey values of DMSP content.

Ackman et al. (1966a) calculated that 10 mg DMSP/d was the minimum intake necessary to deposit a significant level of DMSP in cod flesh and that this value could be reached by fish feeding exclusively on *L. helicina* with a DMSP content as low as 0.1 mg/g. During our study, the weight-specific DMSP content of pteropods was always higher than 1 mg/g, even in the DMSP-poor Labrador Current waters. Our data suggest that cod eating on average 3400 pteropods per day will ingest enough DMSP to develop the characteristic sulfur smell (Table 3). Given the conditions found during this cruise, this value would decrease to approximately 1800/d in the Labrador Current. Obviously, the minimum amount of ingested pteropods required to produce the sulfurous condition will decrease significantly during a bloom of a DMSP-producing alga. Results from station 6, where we measured high DMSP concentrations, indicate that the minimum daily ingestion rate of pteropods by cod could be as low as 680 (Table 3). The transfer of DMSP to *L. helicina* from DMSP-producing algae may thus explain the recurring nature of the blackberry feed problem in the Canadian East Coast waters. Sufficient DMSP accumulation in the pteropods and the appearance of the blackberry symptoms in cod do not necessarily require a bloom of strong DMSP producers. Internal levels of DMSP in pteropods with diameters sufficient to attract cod are always apparently sufficient to initiate the blackberry feed problem. The key factor appears to be the abundance of pteropods, with sufficient DMSP, serving as the major food supply to cod. This triggering concentration will change based on the diet of the pteropods and their interannual population dynamics. The feeding enthusiasm of cod for such relatively small organisms as *L. helicina* could be explained by observations on the role of DMSP as a feeding attractant (Nakajima et al. 1989).

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Effect of Coastal Winds on the Summer Dispersal of Young Least Cisco (*Coregonus sardinella*) from the Colville River to Prudhoe Bay, Alaska: A Simulation Model

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A one-dimensional advection model was used to simulate the movement of young (<180 mm) anadromous least cisco (*Coregonus sardinella*) along the Beaufort Sea coast between the Colville River and Prudhoe Bay, Alaska. Two versions of the model simulated eastward movement during the month of July as the sum of wind-induced transport and constant dispersion. Model results were compared with fyke-net catch data collected from 1981 to 1992. Of the 12 years examined, the models correctly simulated no major arrival of fish in 1983, 1985, 1986, 1987, and 1992, simulated the exact day of arrival in 1981, 1989, and 1991, and were in error by 1 d in 1988. Approximately 71% of the error between simulated and observed arrival dates was associated with three specific years: 1982, 1984, and 1990. The model suggests that wind-governed circulation may reasonably account for the presence of young Colville River least cisco in the Prudhoe Bay area. Results are discussed in terms of their relevance for assessing the effects of Arctic oil development on regional fishery resources.

Un modèle d'advection à une dimension a été appliqué à la simulation du déplacement de jeunes ciscos sardinelles (*Coregonus sardinella*) anadromes (<180 mm) le long du littoral de la mer de Beaufort, entre la rivière Colville et Prudhoe Bay, Alaska. Deux versions du modèle ont servi à la simulation du déplacement vers l'est au mois de juillet, en calculant la somme du transport induit par le vent et de la dispersion constante. Les résultats ont été comparés aux données de prise au verveux qui ont été recueillies entre 1981 et 1992. Pour les 12 années examinés, les modèles ont correctement prévu qu'il n'y avait pas d'arrivée importante de poissons en 1983, 1985, 1986, 1987 et 1992, ils ont simulé la journée exacte d'arrivée en 1981, 1989 et 1991 et ils ont été dans l'erreur de 1 journée en 1988. On a associé à trois années précises, soit 1982, 1984 et 1990, environ 71 % de l'erreur entre les simulations et les dates d'arrivée observées. Le modèle indiquerait que la circulation gouvernée par le vent peut raisonnablement bien expliquer la présence de jeunes ciscos sardinelles de la rivière Colville dans la région de Prudhoe Bay. On examine dans quelle mesure les résultats se prêtent à l'évaluation des effets de la mise en valeur du pétrole du l'Arctique sur les ressources halieutiques de la région.

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Anadromous least cisco (*Coregonus sardinella*) have a discontinuous distribution in the Canadian/Alaskan Beaufort Sea (Craig and McCart 1975; Craig 1984, 1989). Western populations are associated with tundra rivers that lie west of, and include, the Colville River, Alaska, whereas eastern populations are associated primarily with the Mackenzie River drainage in Canada. Least cisco are not found in the mountain rivers that lie along the 400 km of coastline between the Mackenzie and Colville rivers (Craig 1989).

The Colville River least cisco population has been studied extensively over the past decade because of its proximity to the oil fields of Prudhoe Bay, Alaska (Fig. 1). As part of ongoing petroleum exploration and production activities, solid-fill gravel causeways have been constructed along the Beaufort Sea coast, and there is concern that these structures, which extend several kilometres seaward from the shore, may disrupt the alongshore summer feeding migration of Colville River fish. There are indeed indications that the West Dock Causeway located just west of Prudhoe Bay may

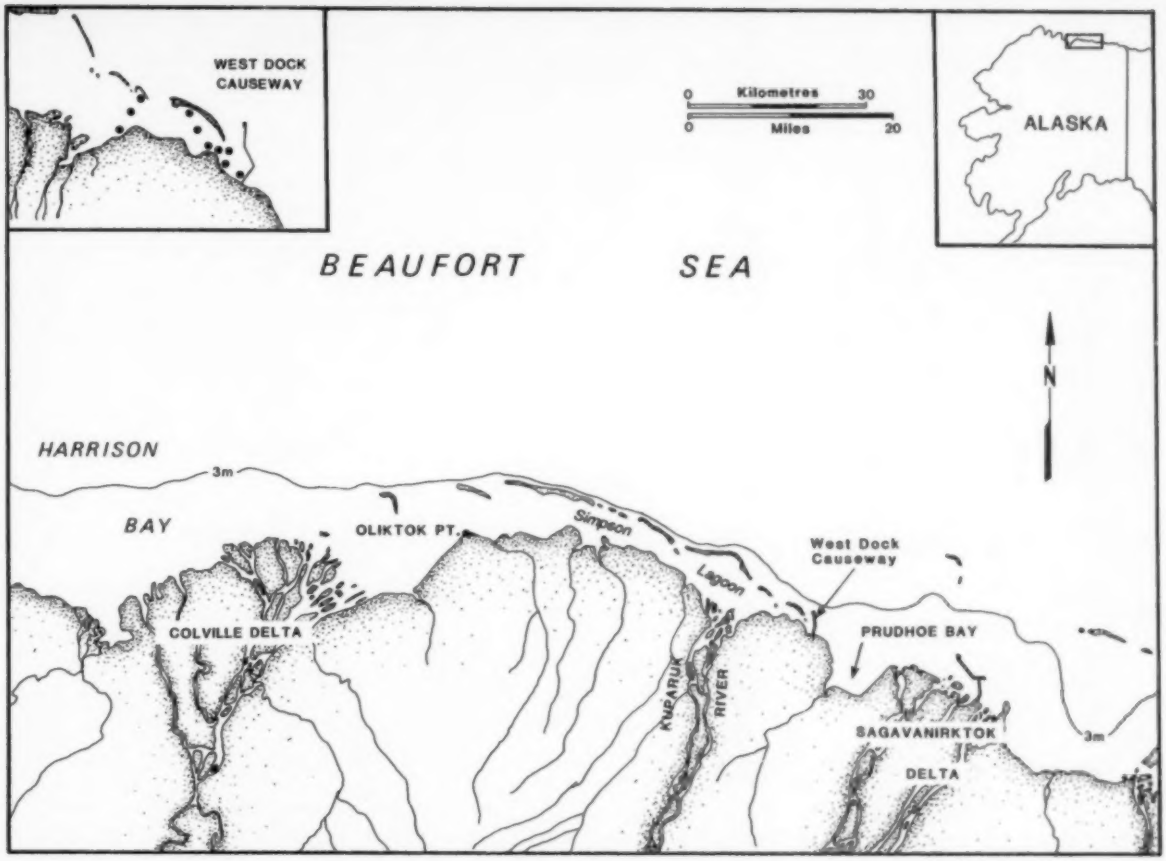

FIG. 1. Western Beaufort Sea coast from the Colville River to Prudhoe Bay, Alaska. Upper left inset denotes fyke-net locations west of the West Dock Causeway. Not all sites were fished in each year.

occasionally disrupt the eastward dispersal of young least cisco by altering local hydrography (Fechhelm et al. 1989; Gallaway et al. 1991; Hachmeister et al. 1991; Robertson 1991). Prolonged east winds cause an eddy to form in the causeway's lee which promotes the vertical mixing of cold, saline bottom water into an otherwise warm and brackish nearshore zone (Niedoroda and Colonell 1990). The eastward dispersal of 1- and 2-yr-old (<180 mm) least cisco appears to be occasionally blocked by this cell of saline water presumably because of their intolerance to high-salinity conditions (Fechhelm et al. 1989).

Assessing the effect of blockage on the overall health and stability of the least cisco population has been confounded because in some years, young fish do not reach the West Dock area in large numbers and are therefore not affected by a wake-eddy (Cannon et al. 1987a; Fechhelm et al. 1989; Glass et al. 1990; Reub et al. 1991). Fechhelm et al. (1989) suggested that these low-abundance years may be linked to wind-driven circulation processes. In late June and early July, waters of the barrier-island lagoon system (Simpson Lagoon) that extends from the Colville River to Prudhoe Bay are typically warm and brackish (i.e., <15‰) because of extensive spring river runoff and the melting of landfast ice (Hachmeister et al. 1991). West winds generate eastward currents in Simpson Lagoon and cause downwelling of marine bottom waters along the coast (Niedoroda and Colonell 1990). This latter process, when coupled with the eastward deflection of plumes from the Colville and Kuparuk rivers, serves to maintain the warm, brackish, easterly flowing character of the lagoon system which, it is hypothesized, enhances the eastward dispersal of small least cisco. In contrast, east winds generate westward currents and cause cold, high-salinity, bottom marine water to upwell into Simpson Lagoon through barrier-island inlets (Fig. 1). The combination of westward currents and the more marine conditions that occur under east winds is thought to impede the eastward dispersal of fish through Simpson Lagoon. While these circulation processes can explain certain patterns in the abundance of least cisco in the Prudhoe Bay area, over a decade of environmental studies have provided mixed evidence in support of the wind-transport hypothesis.

This paper presents a deterministic advection model that simulates the dispersal of young least cisco from the Colville River eastward to the West Dock Causeway for the summers of 1981–92. Modeled results are compared with actual catch/abundance data collected as part of ongoing environmental monitoring programs. Simulations for 1992 represent the first independent application of the model (model parameters are based upon 1981–91 data). Our model lends support to the hypothesis that a wind-governed transport

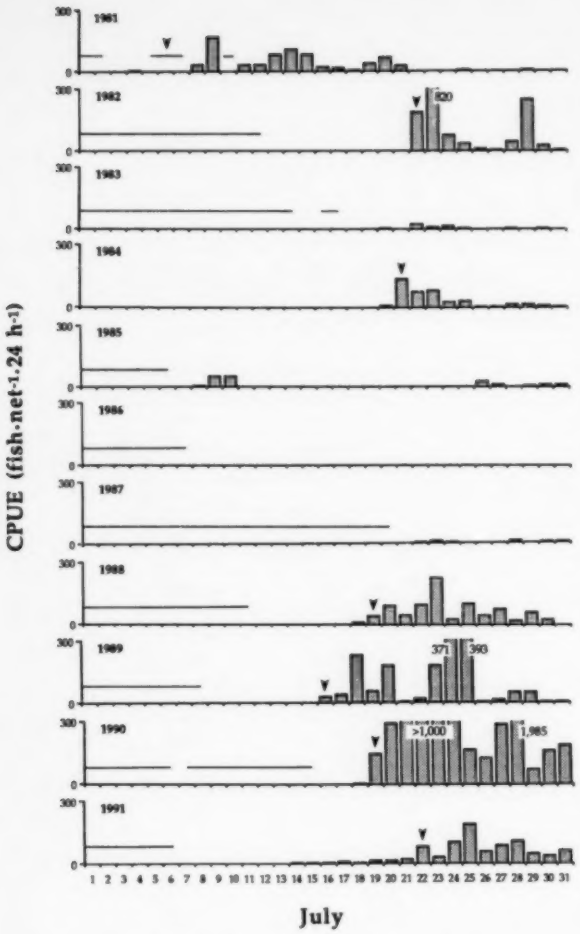

FIG. 2. Mean CPUE of least cisco in fyke nets located immediately west of the West Dock Causeway by year. Horizontal lines indicate periods of no sampling effort. Arrowheads denote arrival date. Simpson Lagoon runs west from the West Dock Causeway to Oliktok Point.

process can reasonably account for the presence/absence of young Colville River least cisco in the Prudhoe Bay area. It also demonstrates how meteorological conditions can govern, and be used to predict, the susceptibility of local fish populations to Arctic oil development.

Methods

The movement of small (<180 mm FL) least cisco was simulated using a point-discharge, one-dimensional (east/west), dispersion/advection model. The model operates under the premise that the net eastward movement of fish through Simpson Lagoon from the Colville River to West Dock is the result of active dispersion coupled with passive drift that occurs in response to wind-induced coastal water currents. The model does not compute the movement of individual fish, but instead calculates the location of the eastern margin of the least cisco's coastal distribution as a single point within Simpson Lagoon. The change in location of the eastern margin in response to active movement is unidirectional (eastward only) and reflects eastward dispersal away from the point of origin (Colville River). Passive movement in response to drift is bidirectional, with fish being transported either eastward or westward within Simpson Lagoon in accordance with wind/current direction. For any unit of time, net movement is the sum of active and passive displacement. The model is described by the equation

$$X(t_N)=\sum_{i=1}^{N}[f\cdot s(t_i)\cdot\cos(\theta_i+c)+d]\Delta t$$

where $X(t_N)$ is displacement (kilometres) from the Colville River, after N time increments, θ_i is wind direction in angular coordinates at $t = t_i$, $s(t_i)$ is wind speed (kilometres per hom) at $t = t_i$, c is the angular deviation of the shoreline from the east/west geographic axis, f is the ratio of wind-induced current speed to wind speed, d is the slack water dispersal speed (kilometres), and Δt is the change in time (hours).

The passive transport component of the model was calculated from wind measurements taken at Deadhorse Airport, Deadhorse, Alaska. Hourly estimates of current speed and east/west current direction within Simpson Lagoon were computed from hourly wind vectors with current speed designated as some fraction f of wind speed. Current speed f was initially estimated at 3% of wind speed based upon observational oceanographic and meteorological evidence (Aagaard 1984; Kozo 1984). Because the angle at which winds intersect the longitudinal axis of Simpson Lagoon can substantially affect the net flow characteristics of the system, the model also compensates for the angular deviation (c) of the shoreline from the east/west geographic axis. While the configuration of the coastline between the Colville Delta and West Dock Causeway is that of an arc, we based our initial estimate of 24° upon the eastern length of Simpson Lagoon nearest West Dock (Fig. 1). Thus, the hourly east/west advective displacement of fish can be summed to give their position within Simpson Lagoon at any time t_i for scenarios using different combinations of f and c.

The period 1–31 July was considered the principal time frame for testing the wind/transport hypothesis. Events prior to 1 July were considered inapplicable to the exercise because Simpson Lagoon is often congested with ice throughout most of June, a situation that would severely bias any wind/current relationship. Events after 31 July were also excluded, since least cisco begin migrating back to the Colville River by early August (Fechhelm et al. 1989; Gallaway et al. 1991).

The displacement constant d represents dispersal from the point of origin independent of a transport process. An initial estimate of d was based upon the observation that in 1989, small least cisco arrived in the West Dock study area on 16 July and that the net east/west wind vector for 1–16 July was approximately zero. Therefore, with no net passive transport component, fish traversed the 80 km between the Colville River and West Dock in 16 d, yielding an average speed of 0.2 $km \cdot h^{-1}$.

Mean catch-per-unit-effort (CPUE, fish per net per 24 h) of least cisco (<180 mm FL) collected in fyke nets located immediately west of the West Dock Causeway (Fig. 1) was used as a measure of the model's performance and to find optimum parameter values. The arrival of least cisco from the Colville River typically appears as a prominent increase in CPUE at these nets (Fechhelm et al. 1989). Because catch

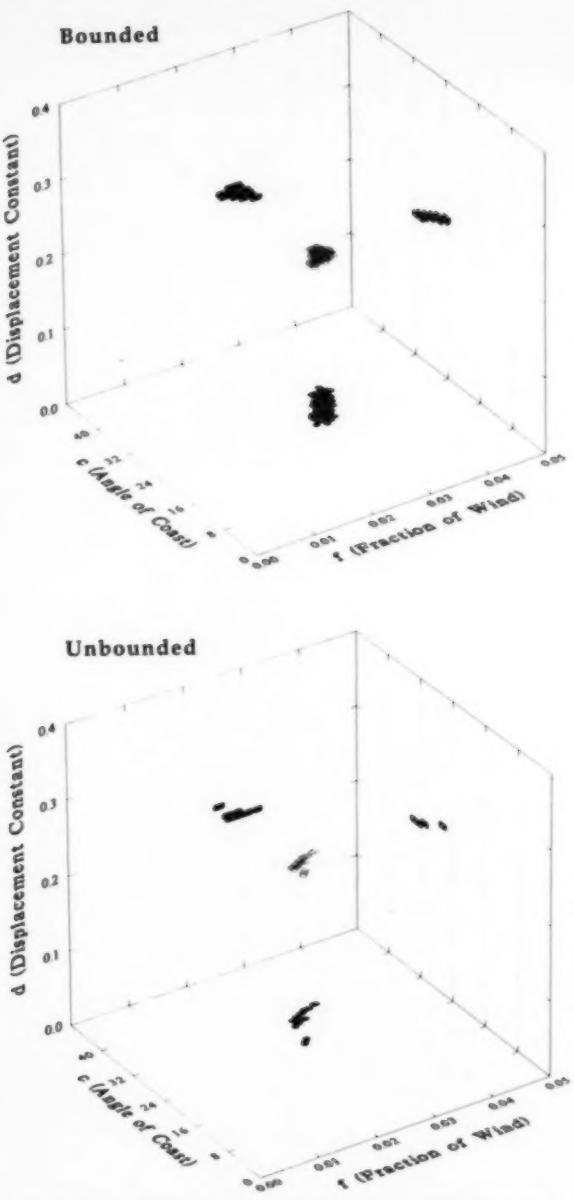

FIG. 3. Three-dimensional shadow projections which show simultaneously the minimum-error parameter estimates for the bounded and unbounded models.

rates for passive fishing gear can fluctuate dramatically for a variety of reasons (e.g., heterogeneous distribution and/or pulsing of fish along the shore, shifting fine-scale hydrographic patterns, changing bathymetric features, inequality of fishing effort due to weather condition, etc.), we used a broad-scale presence/absence criterion for estimating the date of arrival of fish at West Dock. Catch data were plotted for each year and the arrival date determined by inspection based upon mean daily CPUE (Fig. 2). All arrival dates were defined a priori and were not changed to improve the fit of the model. Because of interyear differences in study scope, not all locations were sampled each year. However, since the arrival of fish in the study area generates a conspicuous increase in CPUE at most fyke-net sites, and since a general presence/absence criterion was being used, we felt that the available sampling sites would provide an adequate estimate of relative abundance of least cisco at the eastern end of Simpson Lagoon.

TABLE 1. Simulated and observed arrival dates by year of young least cisco to the Prudhoe Bay area, Alaska, from the Colville River. No sampling was conducted during 1976–80.

	Predicted		
Year	Bounded model	Unbounded model	Observed
1976	No arrival	No arrival	—
1977	No arrival	No arrival	—
1978	No arrival	No arrival	—
1979	23 July	22 July	—
1980	No arrival	No arrival	—
1981	6 July	6 July	6 July
1982	24 July	28 July	22 July
1983	No arrival	No arrival	No arrival
1984	17 July	19 July	21 July
1985	No arrival	No arrival	No arrival
1986	No arrival	No arrival	No arrival
1987	No arrival	No arrival	No arrival
1988	18 July	18 July	19 July
1989	16 July	16 July	16 July
1990	23 July	24 July	19 July
1991	22 July	22 July	22 July
1992	No arrival	No arrival	No arrival

Although most arrival dates were easily determined, the arrival date in 1981 was estimated to have been 6 July, a period of no sampling. Few fish were caught on 3–4 July, yet CPUE increased to 30 and 160 fish·net^{-1}·24 h^{-1} when sampling was renewed on 9–10 July (Griffiths and Gallaway 1982). The midpoint of the intervening nonsampling period was therefore estimated as the arrival date. The years 1983, 1985, 1986, and 1987 were designated as "no arrival" years because of negligible CPUE (Fig. 2). These low catch rates were confirmed by low CPUE at fyke nets located east of the West Dock Causeway (Cannon et al. 1987a; Glass et al. 1990; Reub et al. 1991). Because no major arrival could be detected, these four years were designated as "no arrival" years.

Model simulations were run for different combinations of d (dispersal speed, kilometres per hour), c (angular deviation of the shoreline from the east/west geographic axis), and f (fraction of wind speed). Designated parameter values were held constant throughout each 11-yr set of simulations. For each yearly simulation, fish began at the Colville River on 1 July and their position along the 80-km distance to West Dock was updated every hour using the appropriate values. Arrival at West Dock was designated as the day on which the net distance traveled from the starting point first reached 80 km. For each year, error was defined as the number of days difference between the simulated arrival date and the actual arrival date. If fish arrived in years in which no actual arrival was observed (1983, 1985, 1986, and 1987), the error was calculated from 1 August. Errors for all

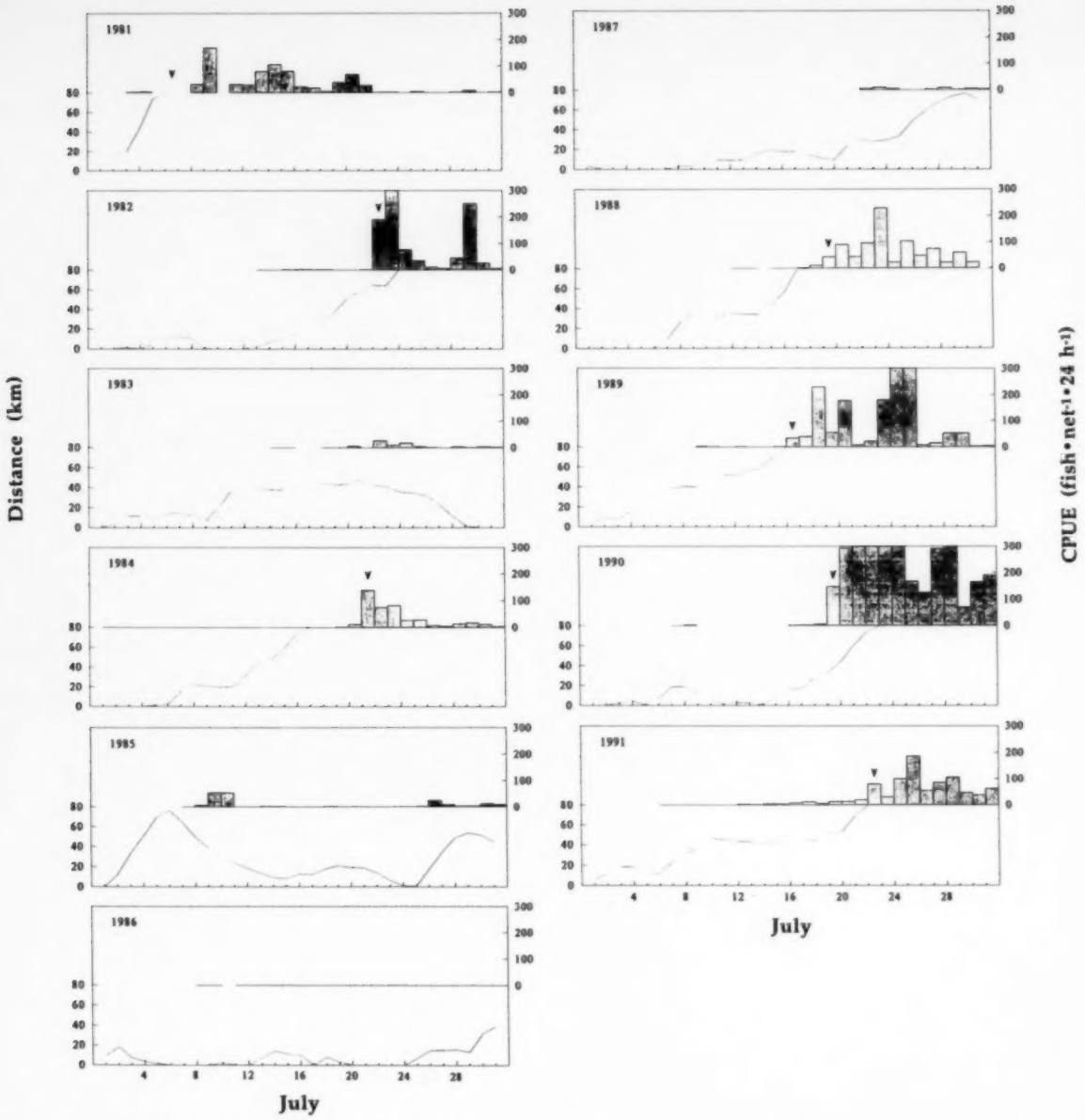

FIG. 4. Simulated distance traveled from the Colville River by year for the bounded model using minimum-error parameter estimates of d (dispersal speed, km·h^{-1}) = 0.20, c (angular deviation of the shoreline from the east/west geographic axis) = 28°, and f (fraction of wind speed) = 0.031. The distance line terminates if net distance traveled reaches 80 km prior to 31 July. Histograms represent observed daily mean CPUE for each year. Absence of CPUE baseline indicates no sampling effort. Arrowheads denote original arrival date estimates.

11 years were summed to yield total error for any given combination d, c, and f. Minimum-error parameter estimates represent those estimates of d, c, and f that minimize the total error for the 11-yr data set from among the 96 000 combinations that were tested.

Two variations of the model were investigated: unbounded and bounded. In the unbounded model, fish are allowed to move either east or west of the Colville River. The underlying assumption is that the quality of the entire coastal feeding habitat is relatively homogeneous for small least cisco and that fish may move freely eastward into the sheltered waters of Simpson Lagoon or westward along the exposed coast of Harrison Bay. In the bounded model, fish are allowed to move anywhere along the 80-km transect between the Colville River and West Dock but are not permitted to move west of the Colville River. The underlying assumption here is that the exposed coastal areas of Harrison Bay are colder and more saline than the Colville Delta/Simpson Lagoon

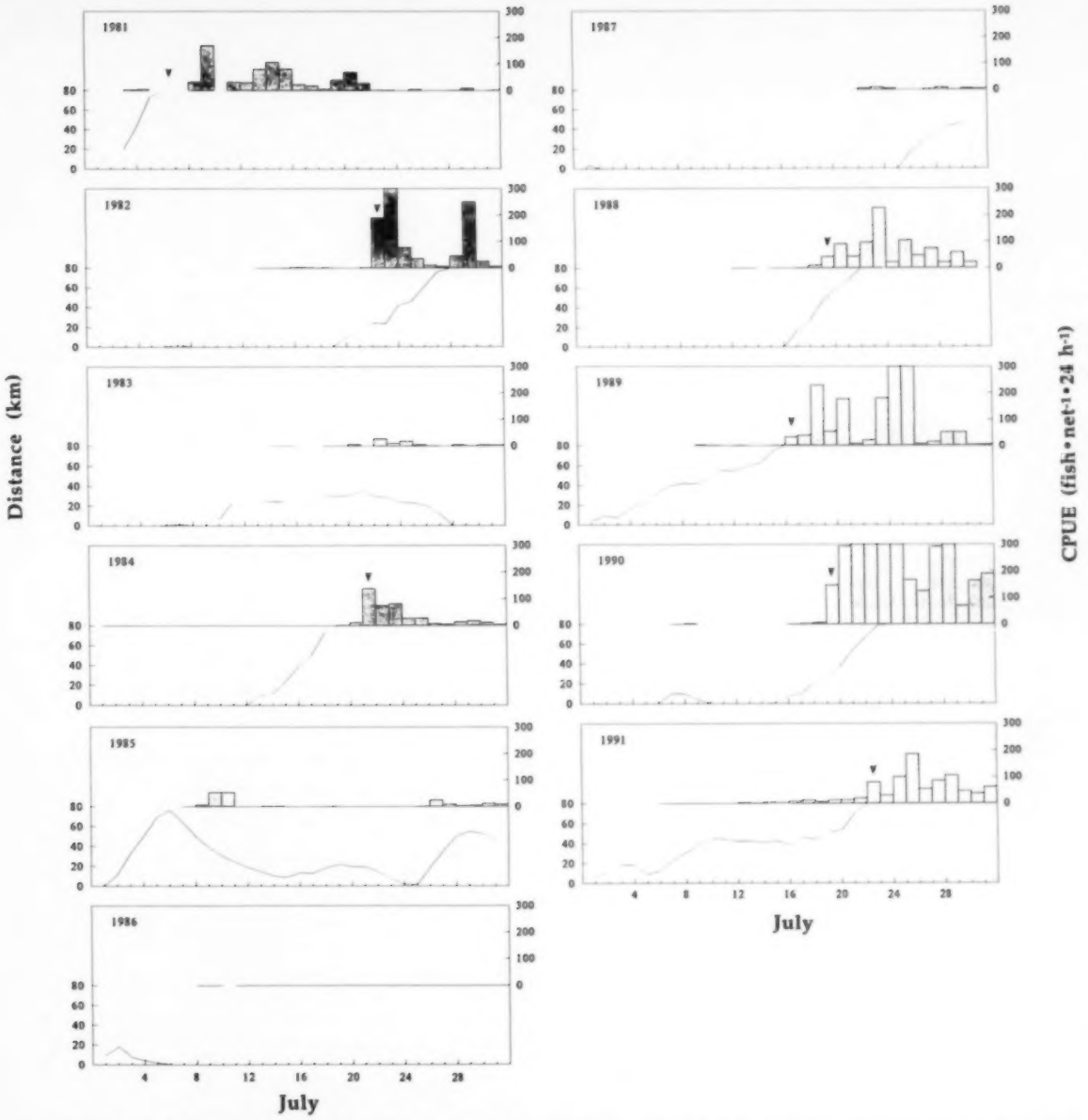

FIG. 5. Simulated distance traveled from the Colville River by year for the unbounded model using minimum-error parameter estimates of d (dispersal speed, km·h^{-1}) = 0.21, c (angular deviation of the shoreline from the east/west geographic axis) = 31°, and f (fraction of wind speed) = 0.027. The distance line terminates if net distance traveled reaches 80 km prior to 31 July. Histograms of observed daily CPUE are superimposed on each yearly plot. Absence of CPUE baseline indicates no sampling effort. Arrowheads denote original arrival date estimates.

area and, therefore, represent less than optimal feeding habitat. Much of the exposed Harrison Bay coast is outside the influence of the Colville River plume and subject to unsheltered marine upwellings during east winds. The bounded model examines the extreme case that least cisco are completely restricted from moving into Harrison Bay, in a sense treating the region of the Colville Delta as a constant source of fish. The purpose for exploring the two alternatives was the hope that disparities in results might shed some light on the regional distribution dynamics of the Colville River least cisco population.

Results and Discussion

Model fit using 96 000 parameter combinations (d: 0.00–0.40 km in 0.01-km increments; f: 0–48° in 1° increments; c: 0.00–0.50 in 0.01 increments) yielded total errors that ranged from 11 to 175 d for the bounded model and from

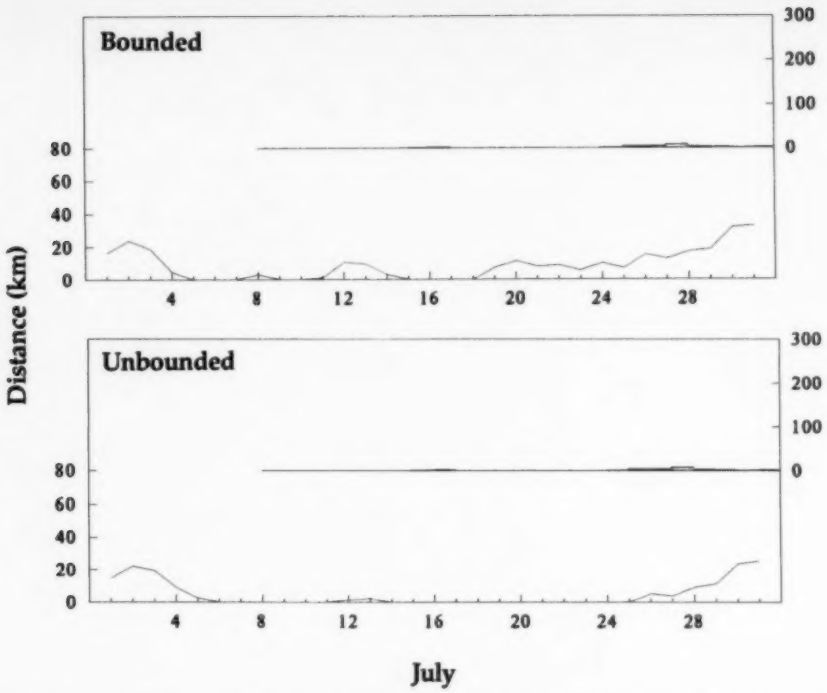

FIG. 6. Simulated distance traveled from the Colville River during 1992 for the bounded (d = 0.20, c = 28°, and f = 0.031) and unbounded (d = 0.21, c = 31°, and f = 0.027) models with histograms of observed daily CPUE. See Fig. 4 for further explanation.

14 to 170 d for the unbounded model. Parameter values that yielded a minimum total error of 11 d for the bounded model (i.e., minimum-error parameter estimates) ranged from 0.19 to 0.21 km for the displacement constant d, from 24 to 32° for the coastline angle c, and from 0.027 to 0.033 for the fractional wind component f (Fig. 3). Minimum-error parameter estimates that yielded a total error of 14 d for the unbounded model ranged from 0.19 to 0.21 km for the displacement constant d, from 24 to 32° for the coastline angle c, and from 0.027 to 0.033 for the fractional wind component f (Fig. 3). From these data the median minimum-error estimates were estimated as d = 0.20, c = 28°, and f = 0.031 for the bounded model and d = 0.21, c = 31°, and f = 0.027 for the unbounded model. These results compared reasonably well with initial estimates of 0.2 km (d), 24° (c), and 0.030 (f).

The bounded and unbounded models were parameterized with their respective minimum-error estimates and then used to simulate the position of fish between the Colville River and West Dock for July of each year (Table 1; Fig. 4 and 5). Both models correctly simulated "no arrival" at West Dock in 1983, 1985, 1986, and 1987, and both correctly simulated arrival dates of 6 July in 1981, 16 July in 1989, and 22 July in 1991. The bounded model simulated an arrival date of 23 July in 1990, which was 4 d later than observed, while the unbounded model simulated an arrival date of 24 July, 5 d later than observed. The bounded model also produced a 4-d error in 1984 when it simulated that fish would arrive on 17 July as opposed to the actual arrival date of 21 July. This compares with a 2-d error generated by the unbounded model in 1984. The largest error was for the unbounded model applied to the 1982 wind record (Fig. 5). The model simulated that fish would arrive on 28 July, 6 d after the observed arrival. The bounded model yielded an error of only 2 d, also late, in 1982 (Fig. 4). Overall, approximately 71% of simulation errors were associated with specific years: 1984 and 1990 (bounded model) and 1982 and 1990 (unbounded model).

The bounded and unbounded models were applied to wind data for 1992 as the first independent application, i.e., minimum parameter estimates were not adjusted for the new data set. Both models simulated that there would be no arrival of small least cisco at the eastern end of Simpson Lagoon (Fig. 6). These simulations were corroborated by the CPUE for 1992 (1.3 fish·net^{-1}·d^{-1}) which was the second lowest in the 12-yr period of record.

It had been hoped that contrasting results from the bounded and unbounded models might offer some insights to the overall dispersal dynamics of the Colville River least cisco population, but such was not the case. The similarity in results from both models undoubtedly reflects some degree of flexibility associated with reliance on three adjustable parameters. Further, the nature of the available data may have obscured any meaningful differences. If wind conditions are such that there is no simulated displacement west of the Colville Delta (unbounded version), then there is no operational difference between the bounded and unbounded versions of the model. This was the case in 1981, 1989, and 1991 when prevailing east winds did not promote movement west of the Colville River (unbounded model) — results of the two models were exactly the same for each of these three years (Fig. 4 and 5). In addition, there was

no measurable way to distinguish between the models in 1983, 1985, 1986, and 1987 beyond the general observation that there was no arrival in those years. Thus, the ability to detect differences between the two model versions was diluted by the nature and pattern of July winds.

The northwest-to-southeast orientation of the Beaufort Sea coast might also contribute to the similarity of results among the two model versions. Northeast winds predominate in our study region during summer (Kozo 1984). Deadhorse wind records confirmed this trend in that of 8928 hourly wind measurements taken during July of each year over the 12-yr period, northeast winds blew 67% of the time. This compares with values of 8% (southeast), 12% (southwest), and 13% (northwest). The ability of northeast winds to generate westward advection, however, is greatly diminished because they blow nearly perpendicular to the coast. In contrast, the ability of northwest winds to generate eastward advection is much greater because they blow parallel to the coastline. As an example, we simulated movement between the Colville River and West Dock using three theoretical wind regimes. For each simulation, $d = 0.20$, $c = 28°$, and $f = 0.031$. Wind speed was held constant at 5, 10, or 15 km·h^{-1} within each trial. Maintaining the 5:1 ratio of northeast to northwest winds, we allowed wind direction to alternate, blowing 1 d from the northwest and 5 consecutive d from the northeast. Results suggest that the advective strength of northeast winds is diminished because of their angle-of-attack on the system (Fig. 7). While there was rapid eastward displacement under west winds, there was no net westward displacement under east winds despite their 5:1 predominance in frequency. In fact, pure northeast winds (45° True) were incapable of offsetting the dispersion component of the model in these theoretical cases. (These examples represent extreme cases, as easterly and southeasterly winds could result in considerable westward advection.) Model results suggest that, overall, the predominant northeast winds of the central Alaskan Beaufort Sea would serve more to halt eastward displacement of least cisco as opposed to "pushing" them back to the Colville River or further westward. Thus, with our models, there is a prevailing bias for Colville River fish to move eastward into Simpson Lagoon and to disperse further eastward than westward. This trend would also help to obscure the differences between the bounded and unbounded scenarios.

The dispersal rate of 0.2 km·h^{-1} corresponds to swimming speeds of 0.31–0.58 BL·s^{-1} for fish ranging from 100 to 180 mm in length. While our estimates are within the limits of sustainable swimming speeds documented for other species, they represent the mean displacement rate, or apparent speed, and are therefore somewhat high in comparison with other reported values (Bernatchez and Dodson 1987). On the other hand, the estimate of 0.2 km·h^{-1} is based upon an assumed distance of 80 km and a start date of 1 July. If least cisco begin entering coastal waters during early breakup, it is possible that dispersal from the Colville River (toward Prudhoe Bay) may begin before 1 July. Craig and Haldorson (1981) reported initial arrivals of least cisco at Milne Point (~45 km west of West Dock Causeway) as early as 29 June. Further, if the decreased advective strength of northeast winds resulting from the northwest-to-southeast orientation of the coast is not fully compensated for in the coastal angle parameter estimates (c), there could be an inherent bias in estimated swimming speed. Equivalent northeast and northwest winds do not negate each other but instead enable net eastern advection, and this advective bias could be partially masked at higher estimates of swimming speed.

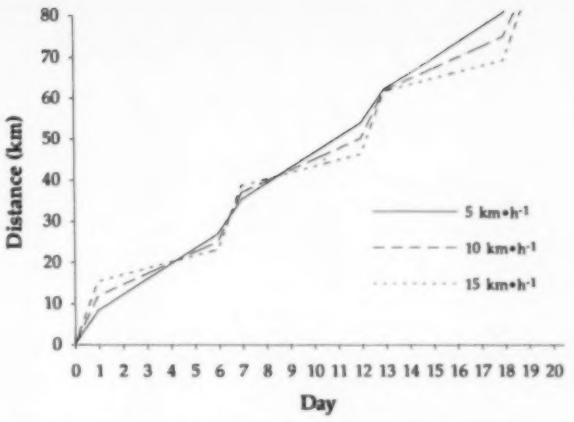

FIG. 7. Simulated distance traveled from the Colville River (0 km) to West Dock Causeway (80 km) under three theoretical wind regimes. For each simulation, $d = 0.20$, $c = 28°$, and $f = 3.1\%$. Wind speed is held constant at 5, 10, or 15 km·h^{-1} within each trial. Maintaining the 5:1 ratio of northeast (315° True) to northwest (45° True) winds that characterize the study region, wind direction alternately blows 1 d from the northwest and 5 consecutive d from the northeast.

While the models are based solely upon advective transport and constant dispersal, other mechanisms associated with wind-driven circulation patterns could conceivably contribute to the east/west movement of fish in Simpson Lagoon. The eastward deflection of the Colville River plume coupled with the absence of marine upwelling under west winds could enhance the dispersal of fish toward Prudhoe Bay by extending a suitable low-salinity habitat eastward. Conversely, the absence of a plume effect and increased marine upwelling into Simpson Lagoon during east winds could result in fish retreating westward to the low-salinity haven of the Colville River delta. The level of food resources in Simpson Lagoon could also influence dispersal. Least cisco rely heavily on marine invertebrates during the summer feeding season (Craig et al. 1984; Moulton et al. 1986; Cannon et al. 1987b; Knutzen et al. 1990; Knutzen and Jewett 1991). Within Simpson Lagoon, a constant replenishment of these marine food items is necessary to maintain the trophic viability of lagoon waters (Craig et al. 1984). The absence of marine upwelling on west winds could impede the influx of entrained marine organisms into the lagoon, while increased upwelling during east winds could enhance the replenishment of prey. Both the hydrographic character of the lagoon and fluctuating prey levels would generally be synchronous with east/west transport and could contribute to the overall dispersal of least cisco along the coast.

Although movement and dispersal are undoubtedly governed by a composite of physical and biological factors, we believe that results of the modeling simulation lend support to the hypothesis that wind-driven circulation processes constitute a major force governing the eastward dispersal of young least cisco from the Colville River to Prudhoe Bay. Data are consistent with observations from temperate

and tropical regions where transport has been implicated as an important factor influencing the distribution and geographic recruitment of larval and juvenile fishes (Kendall and Walford 1979; Baily 1981; Parrish et al. 1981; Smith and Morse 1985; Lough and Bolz 1989; Polacheck et al. 1992; Hare and Cowen 1993). Selective use of transport mechanisms may also be instrumental in the movement and migrations of adult fish (Harden Jones et al. 1979; Arnold 1981; McKeown 1984). What is unique about the Simpson Lagoon system is habitat configuration. Whereas studies of oceanic and tidal transport must consider multidirectional geographic movement coupled, at times, with substantial vertical migrations within a stratified water column, Simpson Lagoon can generally be considered a one-dimensional geographic system (east/west) with limited macrostratification (maximum lagoon depth is ~2 m). Because of their perceived intolerance of high-salinity water, least cisco appear to avoid substantial offshore movement, remaining instead within a narrow coastal corridor. The one-dimensional simplicity of both the physical habitat and the tendency of the fish to remain within its bounds could have rendered the system more amenable to fine-scale modeling efforts.

Evidence of wind-induced transport of young fish along the Beaufort Sea coast is not without precedent. Wind-governed processes appear to play a major role in the dispersal of young-of-the-year Arctic cisco (*Coregonus autumnalis*) from the Mackenzie River in Canada to the major river systems of the Alaskan North Slope (Gallaway et al. 1983; Fechhelm and Fissel 1988; Fechhelm and Griffiths 1990; Schmidt et al. 1991). Interestingly, the meteorogical conditions governing the movement of these two species work in opposite fashion. Summers characterized by strong east winds promote the westward dispersal of Arctic cisco from the Mackenzie River to Prudhoe Bay but impeded the eastward dispersal of least cisco from the Colville River to Prudhoe Bay, and vice versa. Results of this study add to the collective body of evidence which suggests that wind-governed coastal transport processes are key determinants of anadromous fish distribution in the coastal waters of the Beaufort Sea.

The advection model could prove useful in assessing the environmental effects of ongoing and future oil production on anadromous fish stocks of the Beaufort Sea. For instance, the long-term consequences of the West Dock wake-eddy on the overall health of the Colville River least cisco population are undoubtedly linked to degree of susceptibility. Using the complete 17-yr meteorological record (1976–92) from Deadhorse Airport, model simulations were run to estimate the frequency with which small least cisco reach the eastern end of Simpson Lagoon. Both the bounded and unbounded models indicated that there would be no major arrival in 1976, 1977, 1978, and 1980 (Table 1). Overall, no major arrival was simulated for 9 of 17 years (53%), implying that the West Dock wake-eddy would not be a factor in the coastal feeding dispersal of young least cisco in approximately one of every two years. Such estimates, when integrated with other population studies, could provide a framework for making more informed decisions as to the anthropogenic effects of Arctic oil development. Similar extrapolations could be applied to future development along the coast provided that the physical dynamics of site-specific locations are understood.

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An Age-Structured Open-Access Fishery Model

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McGarvey, R. 1994. An age-structured open-access fishery model. Can. J. Fish. Aquat. Sci. 51: 900–912.

A dynamic model for open-access fisheries is presented. In addition to density dependence in recruitment and fishing effort changing in proportion to the level of profit fishermen earn which characterizes previous open-access models, it incorporates full age structure for the fish stock, lognormal environmental recruitment variability, and gear selectivity. The predator–prey cycling solution of the original Schaefer dynamic model, and subsequent open-access models, persists for these model extensions. Density dependence in recruitment induces greater global stability. Environmental recruitment variability, common in marine populations, is destabilizing in the neighborhood of the open-access equilibrium. These two influences, combined in the open-access fishery model, generate robust long-lasting irregular cycles of stock and effort. Volterra proved for the original Lotka–Volterra model that the time averages of the variables over one cycle were exactly equal to their equilibrium steady states. This is shown to extend as a good approximation for the model presented here. Approximating model steady states of effort and catch by the corresponding averages from data time series underlies a new algorithm of parameter evaluation, applied here to an open-access model of the Georges Bank sea scallop (*Placopecten magellanicus*) fishery.

Un modèle dynamique de pêche à accès libre est présenté. En plus du lien de dépendance avec la densité qu'on observe au niveau du recrutement et avec l'effort de pêche qui change en fonction du profit qu'obtiennent les pêcheurs, caractéristiques des modèles précédents à accès libre, notre modèle incorpore une structure complète par âge du stock de poissons, une distribution logarithmique normale de la variabilité du recrutement en fonction de l'environnement et une fonction de sélectivité des engins de pêche. La solution de récurrence prédateur–proie que faisait ressortir le modèle dynamique original de Schaefer, ainsi que les modèles à accès libre subséquents, est conservée avec ces additions au modèle. La dépendance qui existe entre le recruitement et la densité conduit à une meilleure stabilité globale. La variabilité du recrutement en fonction de l'environnement, phénomène commun dans les populations marines, est déstabilisante pour les valeurs qui correspondent grosso modo à l'équilibre en cas d'accès libre. Ces deux influences, qui sont combinées dans le modèle de pêche à accès libre, conduisent à l'apparition de robustes cycles irréguliers et longtemps conservés de stock et d'effort. Volterra a prouvé qu'avec le modèle original Lotka–Volterra, les valeurs moyennes dans le temps des variables au cours d'un cycle étaient exactement égales à leur état stationnaire. Il est montré que cela peut être extrapolé pour constituer une bonne approximation pour le modèle présenté ici. L'approximation des états stationnaires pour l'effort et pour la prise, dans le modèle, par des moyennes correspondantes extraites de séries chronologiques, est à la base d'un nouvel algorithme d'évaluation des paramètres qui est appliqué ici à un modèle à accès libre de la pêche du pétoncle géant (*Placopecten magellanicus*) sur le banc Georges.

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Fishery science is largely founded on two principal classes of models: production models, due to Graham (1939) and Schaefer (1954, 1957b), and the yield-per-recruit theory of Beverton and Holt (1957). Both assume that fishing effort is held constant and derive the level of effort needed to maximize yield. In real fisheries, however, particularly but not only in those free of management restraints, fishing effort varies due to a number of factors. Insofar as fishing is primarily an economic activity from the standpoint of the fishermen, economic factors have been widely assumed to be the principal driving force behind changes in the number of vessels in a fishery (Gordon 1953; Schaefer 1954). Nearly all models that incorporate the change of effort fall into the class known as open-access models, which assume that fishing effort rises when the fishery is profitable and that it declines when, because of declines in stock abundance, fishing costs exceed catch revenues and money is being lost. Models that incorporate this profit assumption express the simplest a priori hypothesis about how effort will change in a fishery with respect to stock abundance, price, and cost.

Schaefer (1954) constructed the first open-access fishery model. The two variables were fish stock biomass and effort, where the latter was understood as the number of (identical) vessels fishing that stock. In Schaefer's dynamic model (1954, equations 12 and 13) (from which the more widely recognized Schaefer production model (1954, 1957b) and its associated paradigm of maximum sustainable yield were derived under conditions of equilibrium and fixed effort), these two variables were dynamic and mutually interacting. Stock biomass rose logistically. This growth of population biomass was balanced by a negative term for the rate of harvest, assumed to be proportional to both fish stock density and the level of fishing effort. The effort equation (Schaefer 1954, equations 10 and 11) postulated a rate of

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change proportional to the total profit fishermen earned. Schaefer (1954) noted that his system of equations was a form of predator–prey model, with effort acting in the dynamical role of predator and fish stock as prey. Intuitively, when stock rises, effort rises, drawn by higher revenues. But high effort, in turn, drives down stock abundance. Effort then declines, allowing the stock to once again repopulate, and the open-access predator–prey cycle begins again. He solved the system analytically (1954) using a linear approximation method of Lotka (1923). Clark (1976) and Wilen (1976), citing Smith (1969), reformulated the open-access model using explicit parameters for the price of fish and cost of fishing. In the economics literature, a theory of "common property", i.e., open-access, dynamic models was developed independently (Smith 1968, 1969, 1971). The models of Fullenbaum et al. (1971, equations 7, 10, 12, and 16) and Leung and Wang (1976, equations 1.5) were mathematically identical to that of Schaefer (1954). Open-access models have been applied to a number of fisheries (Wilen 1976; Bjørndal and Conrad 1987; Gomersall 1992; Sampson 1992). Allen and McGlade (1986) considered spatial movement of vessels and added price as a third dynamical variable.

The predator–prey nature of the open-access model of Schaefer (1954) or Clark (1985) follows from its mathematical form. When logistic prey growth is incorporated in the Lotka–Volterra model, it becomes identical to the Schaefer open-access model, once parameters are lumped and renamed. Visual comparison in the prey–predator phase plane of solutions of the Schaefer dynamic model (e.g., Schaefer 1954, fig. 3; Clark 1985, fig. 1.6) with solutions of the damped Lotka–Volterra model (e.g., Lotka 1924, fig. 14) reveals their common behavior. Both are stable spirals, oscillating in predator–prey cycles while damping down to the steady state. In open-access fishery models, the steady state is the Schaefer open-access equilibrium.

In this paper, the predator–prey nature of open-access fisheries dynamics is investigated. Much is known about the mathematics, in particular the solutions of ecologically derived predator–prey models. This mathematical knowledge will be applied here to the bioeconomics of open-access fisheries.

Schaefer's original model used the single lumped variable of population biomass which he noted to be an oversimplification (1957a). Two subsequent aspects of fishery model construction have provided the basis for a more complete description of the fish stock, namely the incorporation of age structure and stochasticity.

Fish stock age structure, incorporating age-specific gear selectivities and cohort survivals, was developed in the models of Deriso (1980), Getz (1980), Huang and Walters (1983), Swartzman et al. (1983), Schnute (1985), and others, reviewed by Getz and Haight (1989). The age-structure-induced time lags of the fish stock response to harvesting and recruitment are thereby explicit.

Another potentially important influence on fish stock population dynamics is variability in the marine environment affecting yearly recruitment success (Sissenwine 1984; Rothschild 1986). Annual recruitment variability has been shown to satisfy a lognormal distribution in a wide range of fish populations. Hennemuth et al. (1980) found the lognormal to better characterize 15 of 18 marine demersal and pelagic fish stocks. Peterman (1981) found the lognormal to yield the same or better fits than the normal distribution in 11 of 12 salmonid stocks. Modelers have therefore made frequent use of the multiplicative lognormal to describe environmentally driven recruitment variability (Allen 1973; Walters and Hilborn 1976; Hightower and Grossman 1985). A lognormal distribution for recruit per egg is also anticipated on theoretical grounds, being the product of survival probabilities at each stage of early life history (Walters and Hilborn 1976; Peterman 1978; McGarvey et al. 1993).

The model presented below is the first to combine (1) full age structure and (2) lognormal recruitment stochasticity, in the description of the fish stock, with the effort dynamics of (3) open access. Rather than deriving optimums, this model is used to investigate the dynamics of an open-access fishery. The principal goal is to assess whether the predator–prey solution persists when these important features typical of temperate commercial fish populations are incorporated.

To derive parameters of this model from time series data, a new and relatively simple method of parameter evaluation was developed and is presented below. It relies on a property of predator–prey models first proven by Volterra (1927).

If a single form of solution is observed in a wide range of open-access models, we may have a greater expectation of observing that dynamic behavior in real open-access fisheries. Reviewing the literature on the range of open-access models thus far published, and analyzing the solutions for the model constructed below which incorporates a more detailed description of the fish stock population dynamics than previous models, shows that open-access dynamic models do yield a consistent solution, namely predator–prey cycles about the open-access equilibrium. The mathematical basis for the robustness of the predator–prey model solution, formalized in the theorem of Kolmogorov, has been understood in theoretical ecology. This paper seeks to extend this understanding to a class of predator–prey models, i.e., those of open-access fisheries.

This paper is organized in four parts: (1) a generalized age-structured open-access fishery model is constructed, (2) an algorithm for parameterizing this model is presented and applied to data from the Georges Bank sea scallop (*Placopecten magellanicus*) fishery, (3) the underlying assumption of this method of parameter evaluation is tested in a Monte Carlo simulation, and (4) the dynamical solutions of a range of model adaptations are investigated. Because fish population reproduction is the most uncertain and variable process affecting fish population dynamics, I test for the effects of (i) increasing lognormal environmentally driven recruitment variability, (ii) removing stock density dependence, and (iii) three widely applied stock–recruitment relationships on the age-structured open-access model solution.

This model was first constructed to study the cycle dynamics of the Georges Bank sea scallop fishery which is summarized in the Discussion.

Model Equations

The discrete-time model presented below represents six population processes: (1) cohorts decline with a fixed natural mortality and a fishing mortality that varies with effort, (2) slower reproduction at higher stock densities is described by a compensatory density-dependent relationship between annual egg production and age-2 recruitment. (3) recruitment is additionally subject to year-to-year vari-

ability in the marine environment simulated by stochastic sampling from a lognormal distribution for the population growth coefficient representing egg-to-recruit survival of each year class, (4) the catch rate is proportional to both fishing effort and stock density, (5) catchability varies with age due to gear selectivity, and (6) effort rate of change varies in proportion to profit.

A complete list of variables, the input data, and all model parameters are summarized in Appendices 1, 2, and 3. The time-step subscript t denotes simulation variables. Input data time series are identified by the subscript y.

Independent and Dependent Variables

Both the discrete simulation time variable, t, and simulation age, a, are understood to change annually during spawning. Processes that occur continuously throughout year t, such as catch, natural mortality, and change in boat number, are assumed to occur from the spawning season of year t to the subsequent spawning at the beginning of year $t + 1$.

The stock population vector, $\{N_{t,a}\}$, represents numbers of scallops at time of spawning in year t, in each adult age class, $a = 2, 3, 4, \ldots$. Effort, E_t, is the total time, in days, a scallop drag was towed along the bottom during year t (to $t + 1$).

Cohort Survival

For each stock age class, cohort survivors to the subsequent year are calculated as

$$(1) \qquad N_{t+1,a+1} = N_{t,a} - hg_a E_t N_{t,a} - mN_{t,a}.$$

The factor $(hg_a E_t N_{t,a})$ represents the numbers harvested in year t, of age a. The discrete-time catchability coefficient, h, is analogous to the maximum catchability q of continuous-time models. The age-specific commercial gear selectivity vector, $\{g_a\}$, expressing the lower capture rate of younger individuals, rises to a flat asymptote value of 1, for Georges Bank scallops, at age 5. The bilinear functional form of the catch term, proportional to effort and stock, is characteristic of most fishery and predator–prey models including those of Schaefer (1954) and Lotka–Volterra (Lotka 1923). For Georges Bank scallops, this bilinearity was substantiated by the agreement between catch-per-unit-effort (CPUE) and the independent research survey measure of stock size (McGarvey 1991).

The discrete-time natural mortality, m, is assumed constant for scallops of age 2 and up, an approximation satisfactorily corroborated in the field measurements of MacDonald and Thompson (1986).

Density-Dependent Stochastic Stock–Recruitment Relationship

The model measure of parent stock, total annual egg production at spawning,

$$(2) \qquad S_t = \sum_{a=2}^{15} N_{t,a} e_a,$$

and the total biomass of the population,

$$(3) \qquad B_t = \sum_{a=2}^{15} N_{t,a} w_a,$$

are calculated from age-specific vectors of fecundity, $\{e_a\}$, and weight, $\{w_a\}$.

The Shepherd three-parameter stock–recruitment relationship (Shepherd 1982; Garrod 1983) was chosen for its robust form. From this relationship

$$(4) \qquad N_{t+2,2} = \frac{r_t S_t}{1 + (B_t / k)^{\beta}}$$

2-yr-old recruits spawned in year t, $N_{t+2,2}$, are determined, stochastically, each simulation year. The Ricker (1975) and Beverton–Holt (1957) stock–recruitment relationships are also tested in the model. Density dependence in recruitment success is expressed in the denominator. In the numerator, the proportionality, r_t, of eggs, S_t, to recruits expresses an average egg's probability of survival due to external influences in year t.

To simulate the effect of environmental variability on recruitment, r_t is randomly sampled each simulation year from a lognormal distribution with mean $\bar{r}$ and coefficient of variation CV_r. The lognormal distribution, yielding occasional years of very high recruitment interspersed among a majority of relatively low years, typifies fish populations as noted above, and in particular, characterizes Georges Bank scallops. Both recruit numbers (McGarvey et al. 1993) and recruits per egg better approximate a lognormal than a normal distribution by one or more orders of magnitude of significance.

Schaefer Fishing Effort Dynamics

The change in the numbers of vessels and the effort each vessel exerts are hypothesized in an open-access fishery to be driven by the desire for financial gain (Gordon 1953; Schaefer 1954). Thus, fishing effort is modeled to vary in proportion to profit (Schaefer 1954) which is equal to catch revenues minus fishing costs (Clark 1985). The effort model variable, E_t, therefore changes at each time step according to the rate equation

$$(5) \qquad E_{t+1} = E_t + \epsilon \left[\frac{pC_t - cE_t}{cE_t} \right] E_t.$$

Total yearly model catch

$$(6) \qquad C_t = \left[\sum_{a=2}^{15} g_a w_a N_{t,a} \right] hE_t$$

times price, p, yields total yearly revenues which rise in proportion to stock abundance. Likewise, assuming a constant cost per unit effort per year, c, total yearly costs in the fishery, cE_t, are assumed to be proportional to total effort. Therefore the dimensionless expression

$$\left[\frac{pC_t - cE_t}{cE_t} \right] = \left[\frac{p}{c} \cdot \frac{C_t}{E_t} - 1 \right]$$

represents total profit expressed as a fraction of total cost. If $\epsilon = 1$, Equation 5 states that a 10% profit induces effort to increase by 10% per year.

Initialization

The initial values of the variables $\{N_{0,a}\}$ and E_0 were set equal to their estimated steady-state levels. The exception was initial recruitment, $N_{0,2}$, chosen as half the largest age-2 year class in the scallop survey time series, 1980. This initial pulse of higher recruitment acts to perturb the

oscillatory open-access steady state at the outset of each simulation run.

Parameterization

Input data are listed in Appendix 2. Model parameters were evaluated in a six-step algorithm: The values for (1) natural mortality and (2) catchability for use in this discrete-time model were derived from the reported instantaneous (i.e., continuous-time) values, (3) steady-state relative population numbers in each age class (i.e., age structure) were calculated from cohort mortality, (4) absolute model population numbers were obtained from the relative populations derived in step 3 by rescaling with total catch, (5) recruit numbers and egg production from step 4, substituted in the stock–recruitment relationship, permitted the estimation of $\bar{r}$, and (6) price over cost was derived from the time averages of fishing effort and catch. In steps 3, 4, and 6, the steady-state values of model variables were approximated by data time averages.

Step 1

The natural mortality coefficient in this discrete time formulation, m, is calculated from its instantaneous value in continuous time, $M = 0.1$. This estimate was obtained independently in field studies by Dickie (1955), Merrill and Posgay (1964), and MacDonald and Thompson (1986). The instantaneous mortality is defined by

$$N(t+\Delta t) = N(t)e^{-M\Delta t}$$

for all Δt. Setting $\Delta t = 1$ yr, the corresponding difference equation for natural mortality in each age class in each year implies

$$N_{t,a} - N_{t+1,a+1} = mN_{t,a} = [1 - e^{-M}]N_{t,a}$$

yielding

$$(7) \quad m = 1 + e^{-M}.$$

Step 2

Catchability, for this discrete-time model, is calculated from mean instantaneous fishing mortality, F. An estimate of $F = 0.8$ was derived for Georges Bank scallops from the cohort analysis of Robert and Black (1990) by averaging over all years and ages weighted by catch-at-age. Analogous to the result above for natural mortality,

$$(8) \quad h\bar{E} = 1 - e^{-F}$$

yields discrete-time model catchability, h.

Step 3

The steady-state population numbers-at-age are derived from the survival relationship between age classes in a cohort (Equation 1), yielding the stationary age distribution first derived by Lotka (1907):

$$(9) \quad \bar{N}'_a = \bar{N}'_{a-1} - h\bar{E}g_{a-1}\bar{N}'_{a-1} - m\bar{N}'_{a-1}.$$

$\bar{N}'_a$ represents the relative (i.e., unscaled) mean population of age class a. The steady-state-as-average assumption is invoked in substituting the data time average, $\bar{E}$, for the effort steady state. The age-specific commercial gear selectivity vector, $\{g_a\}$, was derived from the scallop dredge gear efficiency studies of Dickie (1955) and Caddy (1972).

The relative population of the youngest age class, N'_2, may be set arbitrarily to a constant. Choosing a value of 1 and iterating (Equation 9) yields $\{N'_a\}$ for older stock ages, $a = 3, 4, 5$, and up.

Step 4

Mean annual catch provides the absolute quantity by which relative populations $\{N'_a\}$ can be scaled to obtain absolute model population numbers $\{N_a\}$, since total catch is proportional to total stock size (Equation 6). However, the mean of catch will retain the same simple bilinear proportionality to the means of stock and effort only if the covariance of stock and effort may be neglected. Cross-correlation analyses of stock and effort, both for Georges Bank scallop time series data and for a theoretical sample of three open-access or predator–prey models, yield values near zero at lag $K = 0$ (McGarvey 1991). Thus, roughly zero covariance characterizes both the real and model predator–grey variable pairs under consideration. Taking time averages in Equation 6, we than have

$$(10) \quad \bar{C} = h\bar{E}\sum_a g_a \bar{N}_a w_a .$$

A scaling factor, α, is defined relating the proportion of relative to absolute population numbers:

$$(11) \quad \bar{N}_a = \alpha \bar{N}'_a.$$

Inserting Equation 11 into 10 yields the estimation formula for the scaling coefficient α:

$$(12) \quad \alpha = \bar{C} \Big/ \left[h\bar{E}\sum_a g_a \bar{N}'_a w_a \right].$$

Step 5

Parameters needed to estimate the Shepherd population growth coefficient are calculated from quantities derived above. Mean stock biomass and mean annual egg production are calculated as

$$(13) \quad \bar{B} = \sum_a \bar{N}_a w_a$$

and

$$(14) \quad \bar{S} = \sum_a \bar{N}_a e_a .$$

Scallop weight as a function of age, $\{w_a\}$, was taken from Serchuk and Wigley (1986). The fecundity with age relationship, $\{e_a\}$, was derived from the field measurements of MacDonald and Thompson (1985; McGarvey et al. 1992) which agree closely with those of Langton et al. (1987; McGarvey et al. 1992).

The parameters β and k in Equation 4 describe the form of density dependence. β determines the steepness of the decline in recruitment due to intraspecific competition at high densities. This decrease in survival of eggs to age of recruitment accelerates when stock biomass exceeds k, the parameter analogous to carrying capacity, chosen as a factor, k', of the observed mean stock biomass, $k = k'\bar{B}$. In the case of Georges Bank scallops, the available stock–recruitment data revealed no observable trend beyond a highly variable but statistically significant linear increase in recruits with egg production (McGarvey et al. 1993). In the simulations of Fig. 3 and the left panels of Fig. 4, values

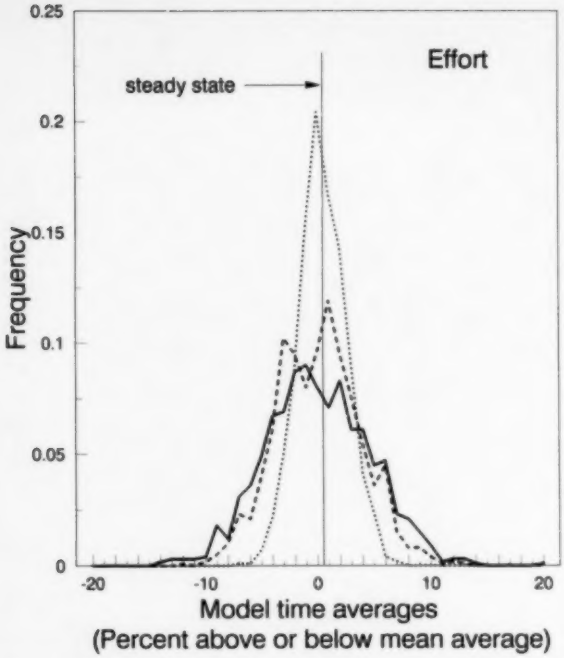

FIG. 1. Frequency histograms of 1000 simulated 30-yr time averages. Zero along the x-axis is the mean effort of 1000 Monte Carlo runs. Histogram partition subintervals are 1% of the effort mean in width. The steady state is the level to which model effort converges after long time periods with no stochastic variation in recruitment. Increasing levels of lognormal recruitment variability are reflected as a wider dispersion of time averages in the three histograms, representing recruitment coefficients of variation of 0.20 (dotted curve), 0.35 (broken curve), and 0.45 (solid curve).

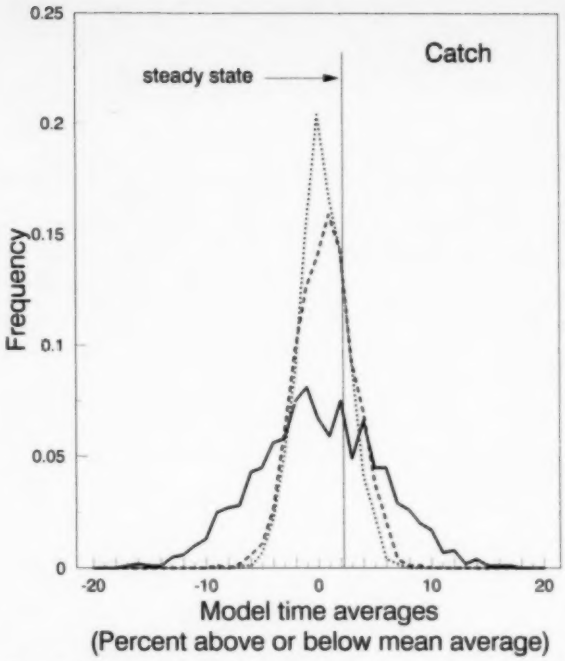

FIG. 2. Histograms of 1000 30-year catch averages compared with the steady state, as in Fig. 1.

of $\beta = 1.6$ and $k' = 2$ were chosen from qualitative criteria. Specifically by analyzing age structure before and after harvesting, it was demonstrated that egg to recruit survival increased significantly in the exploited population (McGarvey 1991), implying the presence of density dependence in the Georges Bank population. However, since no quantitative information is available to distinguish the exact form of density dependence, the sensitivity of the model solution to two alternative stock–recruitment relationships was analyzed. A sample of these solutions is presented in Fig. 5.

Applying the steady-state-as-average approximation a second time, substituting the averages $\bar{N}(2)$, $\bar{S}$, and $\bar{B}$ for their model steady states in the equilibrium form of the Shepherd stock–recruitment relationship (Equation 4) yields the stock population growth coefficient

$$(15) \quad \bar{r} = \frac{\bar{N}(2)[1+(\bar{B}/k)^{\beta}]}{\bar{S}}.$$

Step 6

Using the steady-state-as-average assumption a third and last time in the effort rate of change, Equation 5 yields

$$0 = \epsilon[p\bar{C} - c\bar{E}]$$

or

$$(16) \quad p/c = \bar{E}/\bar{C}.$$

The value for the effort response coefficient $\epsilon = 0.4$ was chosen to match the one-cycle periods of model and data. The 16- to 18-yr cycle period was quantified by peak autocorrelation lags of catch and effort from both simulation output and data time series (McGarvey 1991).

Testing the Assumption of Steady States as Averages

The stable steady state of an open-access model, the open-access equilibrium, is the level to which the variables of stock and effort converge after a sufficiently long time elapses. They are obtained analytically by setting the rates of change equal to zero and solving. In the same early work where he proposed the model (Volterra 1927; Scudo 1971), Volterra proved for the classic purely bilinear Lotka–Volterra model that the continuous averages of the predator and prey variables, as they vary over one or more complete cycles, were exactly equal to their steady-state values. This property of predator–prey dynamics was used to evaluate parameters of the open-access model constructed above.

The principal assumption of the method of parameter evaluation, applied in steps 3, 4, and 6, is that the steady-state values of the model variables of effort and catch are equal to the time averages from data. $\bar{E}$ and $\bar{C}$ are obtained directly from commercial time series by averaging over the years 1958–87, nearly two cycle periods. If it can be shown that the Volterra theorem of steady states equal to averages extends, with a good degree of approximation, to the more elaborate predator–prey model presented here, then that assumption is shown to be implicit in the model itself.

Parameter values derived through this approximation would thus be closely linked to the open-access hypothesis.

Monte Carlo Analysis of the Steady-State-as-Average Approximation

The accuracy of approximating model steady states by model time series averages was tested using a Monte Carlo procedure. Three levels of recruitment variability were investigated, sampling from lognormal distributions of r_t having coefficients of variation of 0.20, 0.35, and 0.45. These span the range of recruitment variability that generated an open-access cycle amplitude (measured by overall standard deviation of CPUE and effort) comparable with what was observed in the Georges Bank scallop fishery. The open-access model simulation was run 1000 times. Each simulation was 30 yr in length. The time averages of effort and catch were calculated from each run.

Results

Monte Carlo Results

The resulting histograms of 1000 Monte Carlo 30-yr averages for catch and effort are illustrated in Fig. 1 and 2. Overall, the deviations between model steady state and mean of 1000 30-yr model averages, for the case of (maximum) 0.45 recruitment variability, were 0.5% for effort and 2.2% for catch. The simulation averages varied over standard deviations of 4.5 and 5.2%, respectively. Thus, even with this relatively narrow dispersion, the steady states of both variables fell within one standard deviation of the time average means. Such agreement was observed even though the length of the time series in the simulation, 30 yr, chosen to match the data time series did not span two full cycles of 32–36 yr. This asymmetry of trajectories shorter than two complete cycles may explain the slight bias of smaller time averages relative to the steady states.

Open-Access Model Dynamics

Figure 3 illustrates a randomly chosen simulation run. The predator–prey cycle of effort and CPUE is evident. The two large peaks of CPUE are followed by peaks in fishing effort. When effort is high, stock abundance (as CPUE) declines. In the same manner, when stock abundance is low, reduced by fishing, effort in turn declines. Lower effort then allows the stock to recover and the cycle begins again.

Predator–prey cycles appear as counterclockwise orbits in the predator-as-y, prey-as-x phase plane. These appear widely in the literature of both predator–prey ecology (e.g., Lotka 1923) and open-access fisheries economics (e.g., Schaefer 1954; Smith 1968; Wilen 1976; Leung and Wang 1976; Clark 1985; Bjørndal and Conrad 1987; Gomersall 1992; Hilborn and Walters 1992; Sampson 1992). The fish stock population variable leads effort in phase by a quarter cycle, giving rise to this counterclockwise phase plane geometry. In the plots of Fig. 4 and 5, phase trajectories (i.e., model simulation solutions) begin at the steady state, indicated by the "×". However, the age structure of the model fish stock at t_0 is not in a stationary state — the initial large pulse of recruits is not yet evident in the stock measure of total egg production.

Figure 4 demonstrates the effects of both density dependence and environmental recruitment variability on the age-structured open-access model solution. In the left panels of Fig. 4, the parameters were as derived above. In the right panels, the only change was to increase the carrying capacity to 100 times the mean level of biomass ($k = k' B$; $k' = 100$), yielding negligible density dependence at the levels of stock biomass encountered. The smaller amplitudes of the predator–prey cycles in the left panels reveal the effect of reproductive density dependence as a stabilizing influence. The cycle, which steadily dampens to the open-access equilibrium in Fig. 4a, becomes a long-term (probably neutrally stable) cycle in the simulation of Fig. 4b where density dependence has been removed. The initial slight wobble about the counterclockwise trajectory in Fig. 4b represents the transition to a stationary age structure. Comparing Fig. 4c with Fig. 4d and Fig. 4e with Fig. 4f illustrates the same result: stability, defined as the tendency to remain near the open-access equilibrium, is enhanced by density dependence of recruitment to the fish stock.

Descending plots in all panels of Fig. 4 represent increasing levels of annual recruitment variability, quantified by CV_r. The effect on stability is opposite that of density dependence. Whether with density dependence (left panels) or without (right panels), increasing the stochastic variation of the r parameter, representing the survival rate of an average egg, yields greater cycle amplitude and thus lower stability of the open-access equilibrium.

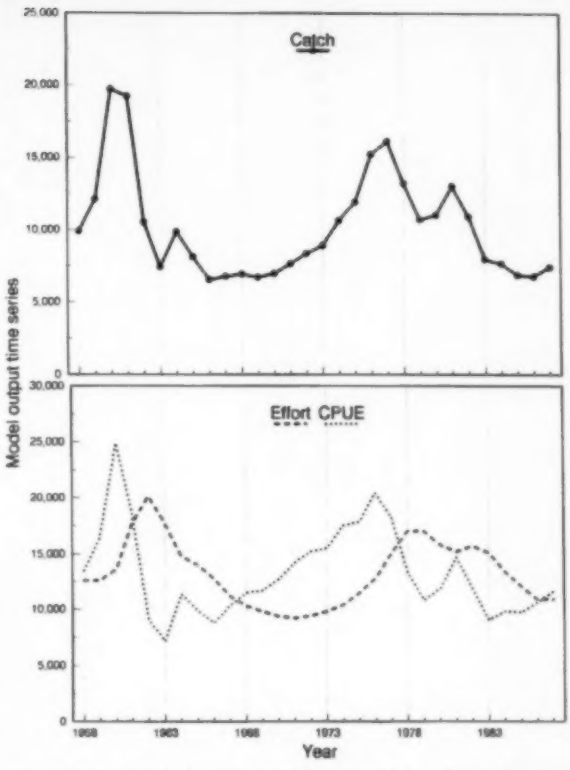

FIG. 3. Open-access model simulation for 30 yr, using parameters and initial values derived in the text. $CV_r = 0.35$. The mean of the CPUE time series was rescaled to equal the mean of effort. Catch is given in units of metric tons of adductor muscle meat. Effort is given in days of dredging time.

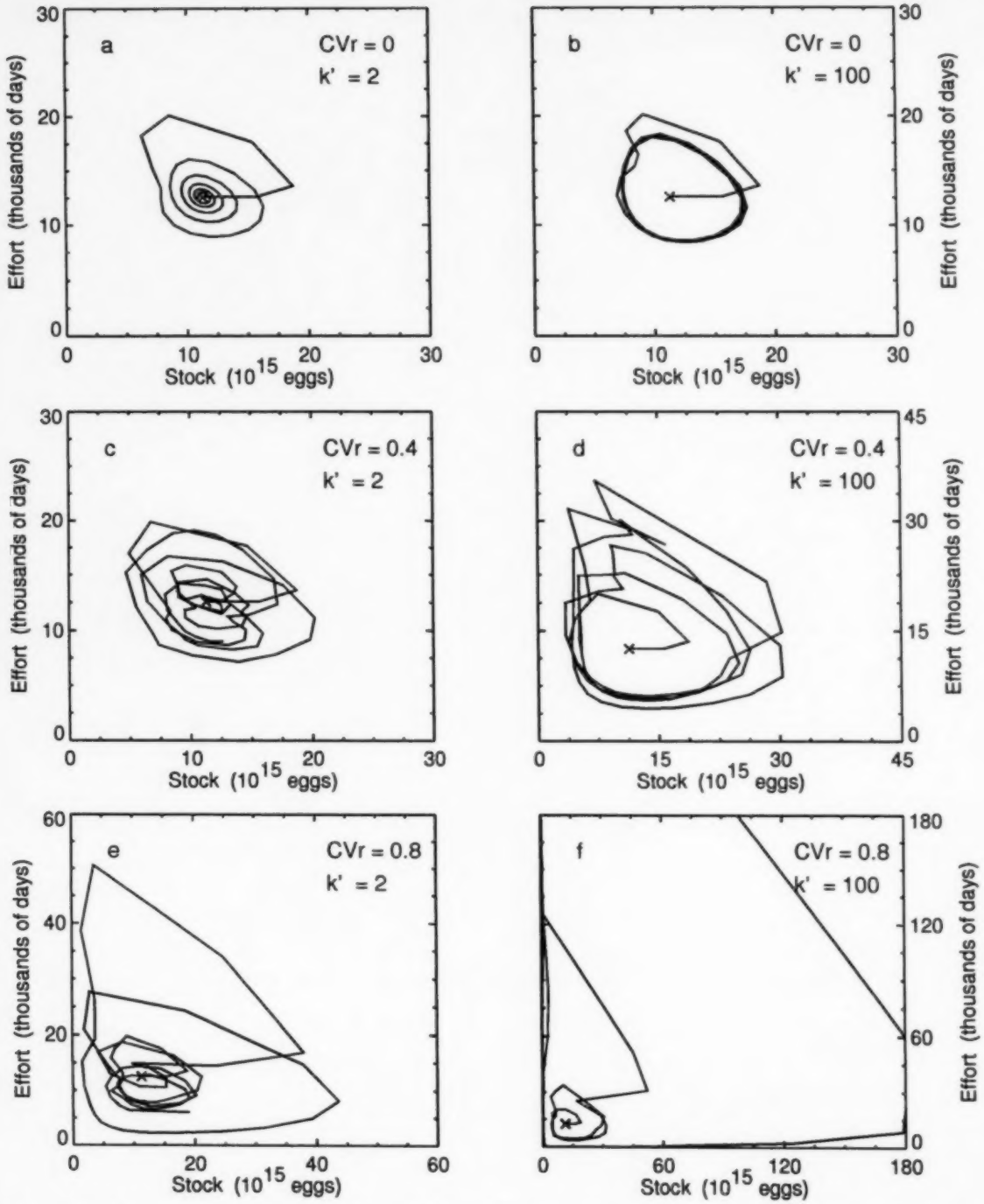

FIG. 4. Phase plot model solutions illustrating the effects of stock density dependence and environmental recruitment variability on open-access fishery dynamics. Recruitment stochasticity increases from top to bottom with CV_r. (a, c, and e) Model with parameter values as derived in the text; (b, d, and f) density dependence suppressed where carrying capacity, k, is set to 100 times the mean level of biomass. Note changes in axis scaling in Fig. 4d–f due to increasing cycle amplitudes.

The model simulation runs in Fig. 5 were undertaken to test the effect of different choices for the stock–recruitment relationship on open-access dynamics. Figure 5a illustrates the Shepherd formula used above. The phase plot of Fig. 5b is thus nearly identical to that of Fig. 4a with one modification: the carrying capacities in the stock–recruitment relationships in Fig. 5 are expressed in units of egg production rather than stock biomass in order to render all three, in standard form, as functions of a single variable. For all three forms of stock–recruitment relationship, open-access predator–prey cycles persist. Stability, however, is affected. The Beverton–Holt (1957) relationship results in greater stability because, in this case, the equilibrium lies in the higher density region of the stock–recruitment relationship. Strong Ricker (1975) density-dependence, where the slope is steeply negative, yields lower stability. This latter extreme form of recruitment relation, decreasing steeply (slope less than -1) with increasing egg production, yields what appears to be a predator–prey limit cycle.

The period of one open-access cycle, identified by autocorrelation analysis (Finerty 1980), was also affected by choice of stock– recruitment relationship. For CPUE, catch, and effort model output time series, the cycle period decreased from 15–16 yr for the Shepherd stock–recruitment relationship to 11–12 yr for the Beverton–Holt form and to 10 yr for the Ricker formulation.

Discussion

This discrete open-access model addresses two simplifying approximations of the original Schaefer (1954) dynamic model noted by Schaefer (1957a): because the fish stock is represented by a single aggregate variable of total biomass (1) the rate of natural stock population increase responds immediately to changes in population density and (2) the rate of natural increase is independent of age structure. By developing age structure for the fish stock, environmental recruitment variability, gear selectivity, fecundity-at-age, weight-at-age, and any desired relationship between recruit numbers and egg production follow naturally in the open-access model formalism. More importantly, time delays between spawning (age 0), recruitment (for scallops, at age 2), and significant contribution to egg production and harvest (ages 4–5) are made explicit.

The results suggest that even complex time lags due to age structure, and high levels of recruitment stochasticity, do not substantially change the basic solution, long recognized for simpler open-access fishery models: open-access predator–prey cycles result.

Cycle stability, however, is sensitive to specific assumptions about fish stock and effort dynamics. Stock density dependence, which by Malthusian mathematical reasoning must characterize all natural populations, increases the rate of convergence of stock and effort to open-access equilibrium levels. The open-access equilibrium may also be made unstable. When annual recruitment is subject to environmental variability affecting the survival rate of an average egg, the open-access cycles spiral away to stock collapse. This effect of prey reproductive variability increasing the instability of the predator–prey cycle solution was first demonstrated in ecological theory context by Nisbet and Gurney (1982) and applied in an open-access fishery model by Allen and McGlade (1986).

In nearly all open-access models reported in the literature, the solution is a stable focus (Schaefer 1954; Smith 1968; Wilen 1976; Leung and Wang 1976; Clark 1985; Allen and McGlade 1986; Bjørndal and Conrad 1987; Gomersall 1992; Hilborn and Walters 1992; Sampson 1992; Hannesson 1993). These predator–prey cycles are damped by the stabilizing influence of stock density dependence.

When density dependence and variability in recruitment success, are combined in an open-access fishery model, long-term irregular predator–prey cycles result. Recruitment stochasticity causes the effort steady state, which is proportional to r_i, to move up and down yearly in the phase plane. This perpetuates the cycles, preventing stock and effort from settling at steady values, since none exist. At larger distances from the steady state (i.e., for larger cycle fluctuations of stock and effort), density dependence acts globally to stabilize the oscillations and to prevent stock extinction. Long-lasting cycles induced by these two processes are illustrated in the simulations of Fig. 3 and 4. Since these two factors are widespread in commercial fish populations, this dynamic may be observable in real open-access fisheries (Allen and McGlade 1986). An open-access interaction with effort can therefore be a plausible hypothesis for observed fish population cycles.

The model presented above was constructed to investigate open access as one of three hypotheses for the apparent cycle of Georges Bank scallops (McGarvey 1991). The model generated time series representing an open-access fishery. These were analyzed to obtain predictions of the open-access hypothesis for spectral time series analyses of the data, including auto- and cross-correlations of stock, effort, and catch. The evidence, in particular the strong similarity in the pattern of the cross-correlations of stock versus effort and also of recruitment versus egg production, applied to both fishery data time series and to the corresponding age-structured open-access model outputs, supported the open-access hypothesis for the cycle.

As a management tool, open-access models may be used to test strategies where the dynamic response of fishing effort is explicit. For regimes that regulate the price of fish or cost of fishing, e.g., by instituting the purchase of a fishing license, where time delays are significant between gathering information about the stock by management and the subsequent setting of new quotas, open-access fishery models can represent the time-dependent response of both effort and fish stock. This was undertaken in a previous study where the ability of various management strategies to achieve the dual goals of maximum yield and stability of the open-access equilibrium, subject to environmental variability as well as birth and death demographic stochasticity of vessel entry and exit and catch, was simulated (McGlade and McGarvey 1992). The model outcome indicated that both objectives, stability and maximum yield, can be attained, after a lengthy transition period, by the classic prescription of fixing effort at a level well below the open-access equilibrium.

Open access also characterizes the dynamics of new fisheries. It is common during the initial development phase of exploitation for effort, and thus catch, to rise to an unsustainable peak, after which catch, and then effort, decline as the stock becomes overexploited. Examples include the North Pacific fur seal (Wilen 1976), Pacific halibut, and California sardine fisheries (Schaefer 1954). The predator–

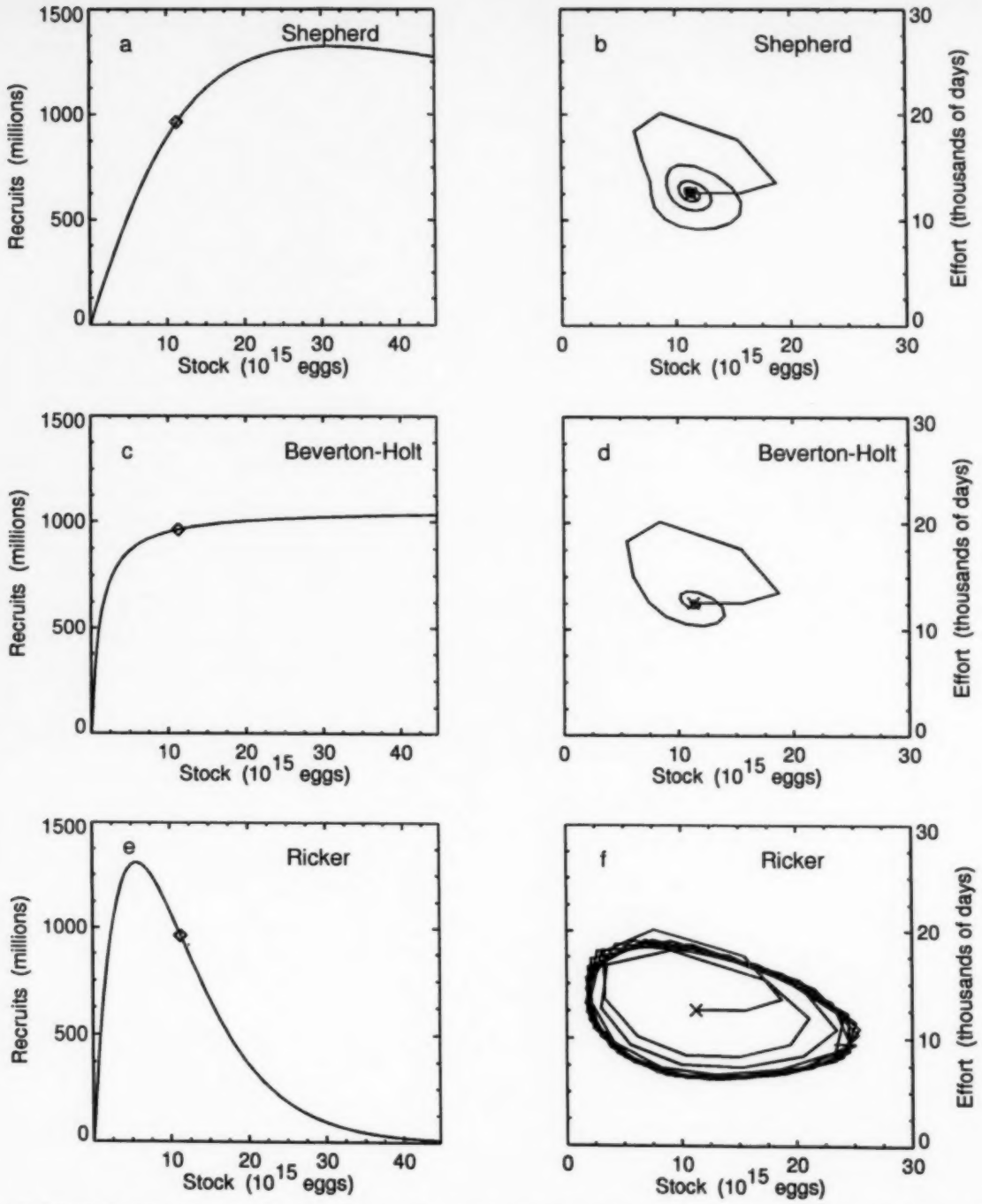

FIG. 5. Testing the open-access model with different stock–recruitment relationships. (a, c, and e) Three relationships between egg production and recruitment discussed in the text; (b, d, and f) corresponding deterministic model solutions; $CV_r = 0$ for all runs. Diamonds indicate open-access equilibrium levels of egg production and recruitment.

prey cyclic trajectories in the effort–stock phase plane spiraling towards a presumed open-access equilibrium (Wilen 1976, fig. 5; Schaefer 1954, fig. 8) were interpreted as expressions of the open-access dynamic. Hilborn and Sibert (1988) warned of unwarranted expectations that this initial peak in catch would be sustainable as a likely cause of mismanagement of new fisheries.

Like open-access predator–prey oscillations, the steady-

state-as-average approximation also appears to be robust. Volterra proved the equality of time averages and steady states for the classic predator–prey model. The Monte Carlo analysis undertaken in this study suggests that the Volterra approximation continues to hold to a 3% accuracy when the pure model is substantially modified. In addition to added features of the open-access model constructed here, including age structure, density dependence, gear selectivity, and recruitment stochasticity, the averages were taken over time series with lengths less than two full cycles. Moreover, this model is formulated in discrete time rather than the continuous time of the Lotka–Volterra model. The steady-state-as-average approximation holds under this set of modifications that are likely to characterize many open-access fisheries. Approximating model steady states by data means offers a straightforward method of parameter evaluation of age-structured open-access and predator–prey models.

Fish population dynamics, especially yield maximums, can be strongly dependent on the form of the stock–recruitment relationship. However, for most commercial fish stocks, no recruitment data are available from the years before exploitation when population densities were high. Data are therefore usually too limited in scope to distinguish among different forms of density dependence. However, the realism of open-access model dynamics is not substantially compromised by inadequate information on the form of the stock–recruitment relationship. In models, as in reality, fishing holds stock population levels well below the carrying capacity. At these ranges of stock density, nearly all common forms of intraspecific density dependence, including the formulas of Ricker (1975) and Beverton and Holt (1957), will simulate, in a similar manner, the tendency for slightly lower egg to recruit survival at the relatively slightly higher stock densities encountered. This would explain why stable predator–prey cycling is observed for all stock–recruitment relationships analyzed in Fig. 5. I did not, however, anticipate that cycles would persist even with the *negative* stock– recruitment relationship of Fig. 5e, since an increase in recruitment with rising stock density (due to lower fishing) is a basic conceptual link in the logic underlying the open-access predator–prey interaction. One intuitive explanation may be offered: the steady-state equilibrium is locally destabilized by the negative stock–recruitment relationship (Ricker 1954; May et al. 1974), and this tendency to spiral away must dominate the predator–prey dynamics as long as stock abundance lies in the range where the Ricker stock–recruitment relationship has a steeply negative slope. When the troughs of egg production reach and fall below this range, i.e., to the left of the peak in Fig. 5e, then the global dynamics once again become stable. The combination of global stability far from the steady state and instability near the steady state generates a limit cycle.

Other modifications of the basic open-access fishery model can yield solutions that are unstable. Clark (1976) investigated the effect of depensatory fish stock growth at low population levels, a variation of the Allee effect, most common with schooling fish stocks such as herring. Clark demonstrated that its effect is destabilizing and under more extreme circumstances can result in catastrophic fish stock collapse.

Other classic predator and prey responses borrowed from ecological theory can be directly applied to their mathematical analogues in an open-access fishery model. A type II predator response (Holling 1959; Begon et al. 1986) occurs when the fish stock is so abundant that the time spent handling, storing, cleaning, or shucking meats reduces effective trawling time. Its effect on open-access fishery cycle dynamics is destabilizing. Fish stock refuges create a type III functional response (Huffaker 1958; Begon et al. 1986), increasing stability of the open-access equilibrium. Nonlinear cost and price relationships can result in multiple steady states (reviewed by Anderson 1986).

The predator–prey literature of theoretical ecology may also be brought to bear on the general question of the robustness of predator–prey cycles as solutions of open-access models. May (1974), invoking the theorem of Kolmogorov (1936; Waltman 1964; Rescigno and Richardson 1967), considered the question of cycling in a broad range of ecologically based predator–prey models. The additions of prey and predator density dependence, predator functional response, and stochasticity do not alter the dynamical solution: virtually all predator–prey models result in predator–prey cycling, either with decreasing cycle amplitude in transitory evolution to a stable steady state or over long times as a limit cycle (May 1974). This mirrors and explains the similarly consistent cycling form of solutions for open-access models in the fisheries literature and in the age-structured model presented above.

The mathematically identical form of dynamical equation for ecological predators and economically motivated fishing vessels under open access is fortuitous. The positive bilinear term in the rate equation represents predator population growth or total fishery revenue. The negative term, linear in the predator, represents predator natural mortality or the cost of fishing.

This predator-style dynamical response of fishing vessels allows for their incorporation in ecosystem models as top predators. They behave mathematically as an additional trophic level. In a conceptual sense, this outcome is compatible with the sociological understanding of fishing as one of the last practices of the hunter–gatherer tradition of substantial economic importance in modern society. This identification of fishing effort as top predator results in a natural model interface between the biology of ecosystem dynamics and the economics of human society.

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Appendices

APPENDIX 1. Summary of model variables.

Variable symbol	Variable name	Description
	Independent (subscripts)	
a	Age	Changes yearly at time of spawning
t	Simulation year	Designates discrete yearly spawning and continuous processes of survival, harvest and effort entry, and exit during t to $t + 1$
y	Data year	Designates input data time series
	Dependent	
$\{N_{t,a}\}$	Population numbers	Fish stock population numbers each simulation year and age
$\{E_t\}$	Effort	Total 24-h vessel days of trawling or active fishing each simulation year
$\{S_t\}$	Parent stock	Equation 2; total yearly egg production by simulation parent stock
$\{B_t\}$	Stock biomass	Equation 3; total simulation population meat weight biomass each year
$\{C_t\}$	Catch	Equation 6; total annual harvest in each simulation year

APPENDIX 2. Summary of input data.

Data variable symbol	Data variable name	Description and reference(s)
$\{C_y; 1958–87\}$	Yearly catch time series data	Reported harvests, U.S. and Canadian fleets from Georges Bank in tons of adductor muscle meat weight (summarized in McGarvey 1991)
$\{E_y; 1958–87\}$	Yearly effort time series data	Reported 24-h days of scallop vessel dredging time (summarized in McGarvey 1991)
F	Fishing mortality, mean	0.8 (Robert and Black 1990)
M	Continuous-time natural mortality	0.1; constant with ages 2+ and time (Dickie 1955; Merrill and Posgay 1964; MacDonald and Thompson 1986)
$\{e_a\}$	Eggs-per-female-at-age	Average annual age-specific fecundity (MacDonald and Thompson 1985; Langton et al. 1987; McGarvey et al. 1992, fig. 2 and table 3)
$\{W_a\}$	Meat-weight-at-age	Age-specific adductor muscle biomass per scallop (Serchuk and Wigley 1986)
$\{g_a\}$	Gear-selectivity-at-age	Derived from empirical studies of commercial scallop gear by Dickie (1955) and Caddy (1972)
	Aggregates	
$\bar{C}$	Average of $\{C_y; 1958–87\}$	Catch time series average
$\bar{E}$	Average of $\{E_y; 1958–87\}$	Effort time series average
	Initial values of the dynamical variables	
$\{S_{0,a}\}$	Stock, initial	Initial recruitment ($a = 2$) age class set to half observed 1980 peak; all other age classes are set to model steady-state values
E_0	Effort, initial	Set to average observed value

APPENDIX 3. Summary of parameters.

Parameter symbol	Parameter name	Description
	Constants	
h	Catchability in discrete time	Derived from reported instantaneous fishing mortality, $F = 0.8$
m	Natural mortality in discrete time	Derived from the instantaneous $M = 0.1$
k	Carrying capacity	Serves role of carrying capacity in Shepherd stock–recruitment relationship
β	Beta exponent	Determines the rate of decline in egg-to-recruit survival, at biomasses > k in Shepherd three-parameter stock–recruitment relationship
ϵ	Effort rate coefficient	0.4; determines the response rate of effort to profit; chosen to yield correct predator–prey cycle period
p/c	Landed price per unit catch by weight/cost per unit effort	Derived using steady-state-as-average assumption in effort rate equation
	Stochastic in time	
$\{r_t\}$	r-Shepherd	Similar to classical "r"; proportionality coefficient between eggs and recruits in Shepherd stock–recruitment relationship; sampled each simulation year from lognormal distribution with mean $\bar{r}$ and CV_r
$\bar{r}$	r-mean	Derived (step 5; Equation 15) using steady-state-as-average assumption
CV_r	Coefficient of variation of r	Quantifies the levels of recruitment variability in the lognormal distribution describing environmentally induced annual fluctuations in egg-to-recruit survival

APPENDIX 3. (*Concluded*)

Parameter symbol	Parameter name	Description
	Intermediates, used in calculations	
$\{\bar{N}'_a\}$	Population, relative mean	Unscaled age-specific mean population numbers; derived from steady-state-as-average assumption in cohort survival rate equation
$\{\bar{N}_a\}$	Population, mean	Absolute age-specific mean population numbers
$\bar{S}$	Stock, mean	Mean annual egg production
α	Alpha	Scaling factor between relative and absolute populations-at-age
k'	k-factor	Proportionality coefficient between k and stock biomass; $k = k'\bar{B}$
$\bar{B}$	Stock biomass, mean	Total meat weight adductor muscle biomass of simulation stock

Gape-dependent Larval Foraging and Zooplankton Size: Implications for Fish Recruitment across Systems

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Bremigan, M.T., and R.A. Stein. 1994. Gape-dependent larval foraging and zooplankton size: implications for fish recruitment across systems. Can. J. Fish. Aquat. Sci. 51: 913–922.

Small gape of zooplanktivorous larval fish limits their prey size; yet, within constraints set by gape, zooplankton size eaten influences larval growth and ultimately survival. To determine if optimal zooplankton size varied among fish species with different gapes, we conducted foraging trials with larval bluegill (*Lepomis macrochirus*, 10–26 mm TL) and gizzard shad (*Dorosoma cepedianum*, 18–31 mm TL). Larvae (n = 10) fed for 1 h on zooplankton assemblages that varied in size, after which all larvae and remaining zooplankton were preserved. Larval gape was measured; both larval gut contents and available zooplankton were quantified. Bluegill, the large-gaped species, fed on larger zooplankton than did gizzard shad with similar gapes. Further, larger bluegill fed on progressively larger zooplankton whereas all gizzard shad ate small prey (<0.60 mm). As available zooplankton size increased, bluegill prey size increased whereas gizzard shad consistently selected small prey. Therefore, differences in zooplankton size among lakes could differentially affect foraging success of larval fishes. In particular, systems with small zooplankton may represent ideal foraging environments for gizzard shad whereas lakes with large zooplankton may favor larval bluegill. If differential larval foraging translates to differential growth and survival, zooplankton size could influence recruitment success and ultimately fish community composition.

La petite ouverture de la bouche chez des larves zooplanctivores de poisson les limite dans le choix des proies que ces larves peuvent ingérer; compte tenu de cette contrainte, la taille du zooplancton qui est ingéré influence la croissance des larves et ultimement leur survie. Pour déterminer si la taille optimale du zooplancton ingéré qui varie d'une espèce à l'autre de poisson en fonction de l'ouverture de la bouche, nous avons procédé à des essais d'alimentation sur des larves du crapet arlequin (*Lepomis macrochirus*, LT 10–26 mm) et de l'alose à gésier (*Dorosoma cepedianum*, LT 18–31 mm). Les larves (n = 10) ont été nourries pendant 1 h avec des groupes zooplanctoniques variables quant à leur taille; au bout de cette période, toutes les larves et le zooplancton qui restait ont été conservés. L'ouverture de la bouche des larves a été mesurée; le contenu stomacal des larves ainsi que le zooplancton ont été mesurés. Le crapet arlequin, l'espèce à la plus grande bouche, s'est nourri d'un zooplancton plus gros que celui des aloses qui avaient une bouche de la même grandeur. En outre, les plus grosses larves du crapet arlequin se sont nourries de zooplancton de plus en plus gros alors que toutes les larves de l'alose ont ingéré des petites proies (<0,60 mm). Plus le zooplancton restant était gros, plus le crapet se nourrissait de proies de plus en plus grosses; l'alose choisissait constamment de petites proies. Donc, des différences d'un lac à l'autre dans la taille du zooplancton pourraient agir différentiellement sur le succès de l'alimentation des larves de poisson. En particulier, les systèmes où on trouve du zooplancton de petite taille peuvent correspondre à un environnement idéal d'alimentation pour l'alose à gésier alors que les lacs qui comptent du zooplancton de grande taille peuvent être plus avantageux pour le crapet arlequin. Si les habitudes alimentaires différentielles des larves se traduisent par une croissance et une survie différentielles, alors la taille du zooplancton pourrait agir sur le succès du recrutement et, ultimement, sur la composition des communautés de poisson.

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Identifying mechanisms underlying fish recruitment ultimately will improve our understanding of community dynamics and patterns of species distributions. However, despite its significance, recruitment historically has been difficult to predict. Recent studies, illustrating the utility of a size-based perspective, demonstrate that many factors affecting survival of larval fishes are size dependent (Forney 1976; Hunter 1981; Post and Prankevicious 1987; Rice et al. 1987; Luecke et al. 1990). Larger larvae tend to be more resistant to starvation, stronger swimmers, less vulnerable to predation (Miller et al. 1988), better foragers (Blaxter 1986; Pepin 1991), and less vulnerable to overwinter mortality (Adams et al. 1982) than their smaller counterparts. Therefore, understanding the processes that underlie larval growth is a critical component of predicting recruitment. Larval growth, in turn, requires successful foraging (Noble 1975; Mills et al. 1989a). Given these linkages among foraging, growth, and size-dependent survival, we may ultimately be able to predict recruitment by identifying ideal foraging environments for larval fish.

Both food quantity and quality drive larval foraging success. Therefore, not only the absolute abundance, but also the

size distribution of zooplankton available to larval fishes influences their foraging success, as well as growth (Arthur 1976; Frank and Leggett 1986; Crowder et al. 1987; Mills et al. 1989a, 1989b; Miller et al. 1990) and survival (Ware 1975, 1977; Frank and Leggett 1986), suggesting that an optimal prey size exists for larval fish. Because previous studies have focused on the relationship between single fish species and zooplankton size, we adopted a multispecies approach to determine if the relationship between zooplankton size and larval foraging is species specific. Further, we evaluated the utility of larval gape in explaining patterns of prey choice.

Larval gape influences larval foraging by constraining the size of zooplankton that larval fish can capture and consume (Wong and Ward 1972; Schael et al. 1991). Therefore, gape should correspond to larval prey size. Because gape varies both within species (through ontogeny) and among species (gape versus length varies with species), it may function as a predictor of prey choice, both within and across fish species. Specifically, differences in gape size between fish species may indicate that they have different optimal zooplankton sizes. Yet, gape alone does not predict prey choice because factors such as visual acuity and foraging mode influence larval foraging as well. Accordingly, within a single system, larvae of different species, with similar gapes, do feed on zooplankton of different sizes (Arthur 1976; Hartmann 1986; Michaletz et al. 1987; Schael et al. 1991). How gape contributes to foraging patterns across fishes requires refinement, for species-specific differences in optimal prey size have important implications for larval success.

Because zooplankton size varies among systems, species-specific differences in optimal prey size could contribute to recruitment patterns (Sprules 1980; Bays and Crisman 1983; Canfield 1988). Whereas some systems are dominated by large zooplankton, such as *Daphnia* or large copepods, others are dominated by small zooplankton, such as *Bosmina* or small copepods. Given the likelihood of species-specific responses to zooplankton size, systems that differ in zooplankton size may represent ideal foraging, and hence recruitment, environments for different larval fishes.

We documented the foraging patterns of two fish species across a gradient of zooplankton size and evaluated the role of gape in larval prey choice. We chose bluegill (*Lepomis macrochirus*) and gizzard shad (*Dorosoma cepedianum*) for these experiments, given that their geographic distributions overlap (Becker 1983), their larvae are zooplanktivorous (Becker 1983), and, as we document below, their relationships between gape and total length differ. Within species, we asked these questions: Do larvae with larger gapes eat larger prey? Does larval prey size change corresponding to available prey size? If so, does an interaction exist between the effects of gape and available prey size on prey choice? Between fish species, we asked: Do larvae with similar gapes eat similar-sized prey? Do larvae respond to zooplankton size similarly? Finally, if species-specific patterns of prey choice were evident, our goals were to (1) evaluate the usefulness of gape as a predictor of prey size and (2) consider the implications of these differences in prey choice to foraging success, growth, and ultimately survival of these two fish species, across systems ranging their geographic distribution, with zooplankton assemblages that differ markedly in size.

Methods

Experimental Organisms

Young-of-year bluegill and gizzard shad were collected from Hebron Fish Hatchery ponds, Hebron, Ohio, during July and August 1990 and held at 23°C in a 14 h light : 10 h dark cycle. Larvae were fed a mixture of brine shrimp nauplii, reservoir zooplankton, and laboratory-cultured *Daphnia magna*. Because fish size ranged 10–26 mm total length (TL) for bluegill and 18–31 mm TL for gizzard shad, both larvae and early juveniles were used in experiments. Hereafter, all fish will be referred to as larvae.

Using a dissecting scope with a drawing tube, we measured larval total length. Larval dry weight of gizzard shad was calculated with a species-specific regression, determined by drying larvae at 80°C for 24 h. A larval bluegill length – dry weight regression was provided by James Breck (Department of Natural Resources, Ann Arbor, Mich.). Larval gapes were measured using a precision gape micrometer, which provides the smallest internal dimension of the mouth (Arts and Evans 1987). This device consisted of stainless steel rods with conical tips that were mounted on a Plexiglas body holding a micrometer scale and measurement head. Gapes of a size-stratified sample of bluegill (n = 39) and gizzard shad (n = 47) were measured. From these gape measurements, species-specific regressions of gape as a function of total length were developed. Gapes for all larvae used in experiments were calculated with these regressions.

Experimental Protocol

Zooplankton used in the experiments were composed of reservoir and hatchery pond zooplankton supplemented by laboratory-cultured *D. magna*. Dominant taxa were *Bosmina* spp., *Chydorus* spp., *Ceriodaphnia* spp., *Daphnia* spp., and calanoid and cyclopoid copepods.

Foraging experiments were conducted in 8.0-L (23°C) containers with opaque sides. To ensure that larval guts were empty at the beginning of an experiment, we starved 10 larvae of a single species overnight by isolating them in covered containers without zooplankton. Each morning, zooplankton were size-fractionated by first straining them through a 180-μm-mesh net. Given their small size, rotifers and copepod nauplii were not included in our experiments. Extremely high densities of these small zooplankton would have been required to provide biomasses comparable with large zooplankton, thus confounding zooplankton size and number across containers. Zooplankton retained by the 180-μm mesh were strained through a 500-μm-mesh net. Zooplankton assemblages were composed of zooplankton (1) retained by the 180-μm mesh but not the 500-μm mesh (small zooplankton), (2) retained by the 500-μm mesh (large zooplankton), or (3) composed of groups 1 and 2, equally mixed (large and small zooplankton). Due to differences in shape among zooplankton taxa, the outcome of size-fractionation varied with taxa. Therefore, rather than creating three distinct zooplankton treatments, this method produced zooplankton assemblages that varied along a size gradient. Each assemblage contained at least 1000 zooplankton ($125 \cdot L^{-1}$); numbers were estimated initially by eye and later quantified.

Experiments began at about 08:00. Lights came on at 07:40; light levels ranged from 215 to 350 lx across con-

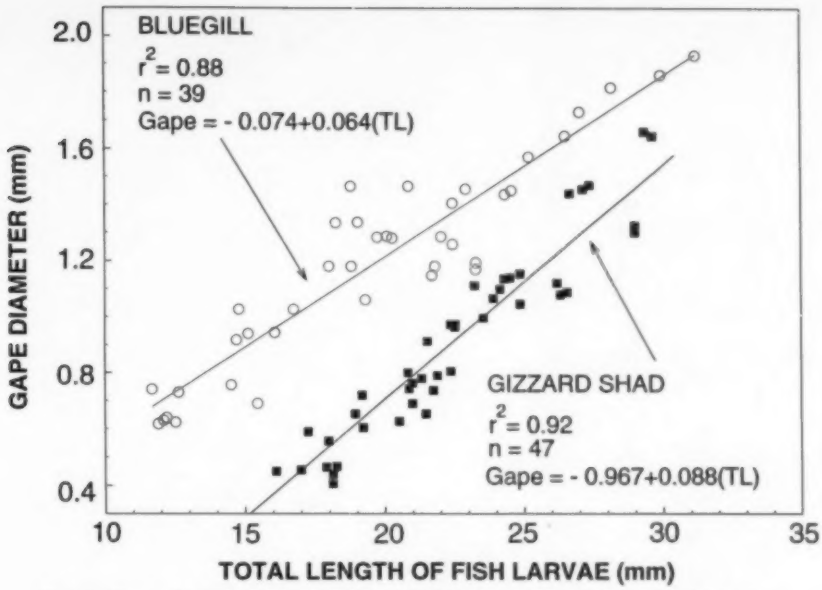

FIG. 1. Gape of larval bluegill and gizzard shad in relation to larval total length.

tainers. At 08:00, covers were removed from the larval fish containers and zooplankton added, with zooplankton assemblages arbitrarily assigned to containers. Larvae fed for 1 h and were then removed and preserved in 10% formalin. Remaining zooplankton were preserved in 5% sugar-buffered formalin. In all, 29 experimental containers contained bluegill; 33 containers contained gizzard shad. Because larvae die if handled, they were not measured before experiments. Therefore, larval size varied both within and across containers. To compare foraging by fishes of a given gape, we considered larvae to be independent units throughout our analysis.

Sample Analysis

To quantify zooplankton consumed by larvae (hereafter referred to as gut zooplankton or prey) and zooplankton remaining in each container at the end of each experiment (container zooplankton), we identified (cladocerans to genus; copepods as calanoid or cyclopoid), counted, and measured zooplankton using a dissecting scope with a drawing tube. For gut zooplankton, the entire digestive tract of each larva was analyzed. In most cases, all prey were measured. In a very few cases, prey were too digested to measure. We assigned measurements to unmeasured prey proportionate to the recorded measurements for prey of that particular taxon in that particular fish. The proportion of prey items in a given fish that were unmeasured never exceeded 50% and rarely exceeded 20%. For container zooplankton, we counted zooplankton from two opposite wedges of a dish divided into 16 equal-sized wedges. From these counts, the total number of individuals within each taxon in the entire sample was estimated. If this number was >25, counting of complete wedges continued until at least 50 individuals of that taxon were counted. If the total number of individuals of a taxon, as estimated from the first two wedges, was ≤25, counting of that taxon stopped because it contributed so little to the entire sample, and the initial estimate of abundance was retained. At least 20 individuals of each taxon were measured (total body length, excluding spines, helmets, and caudal rami). For both gut and container zooplankton, zooplankton lengths were converted to biomass using taxon-specific length – dry weight regressions (G.G. Mittelbach, Kellogg Biological Station, Michigan State University, East Lansing, Mich., unpublished data).

For each experimental container, gut zooplankton were added to container zooplankton to generate estimates of the zooplankton in each container at the outset of experiments (hereafter referred to as available zooplankton). Both average size and number of available zooplankton varied similarly for bluegill and gizzard shad across containers. Average zooplankton size across containers ranged from 0.30 to 1.90 mm; numbers varied from 1000 ($125 \cdot L^{-1}$) to 2600 ($325 \cdot L^{-1}$). Across zooplankton assemblages, average size of available zooplankton tended to decline as number of available zooplankton increased, allowing zooplankton biomass to remain consistent across containers. Therefore, no containers had a high density (i.e., $>300 \cdot L^{-1}$) of large zooplankton (i.e., average size >0.80 mm). At the outset of our analysis, to account for the potential influence of number of available zooplankton, we first tested the influence of both size and number of available zooplankton on larval prey size. Because our analysis did not indicate that number of available zooplankton contributed substantially to patterns in larval prey size, we eliminated it from further analyses.

Results

Larval Gape

To determine if gape size differences were significant between species, we regressed gape as a function of total length and compared a full model (separate regression lines for bluegill and gizzard shad) with a reduced one (one regression line for all larvae). Bluegill had larger gapes than same-sized gizzard shad (Fig. 1; $F_{2,82} = 190.6$, $p \leq 0.001$). Dif-

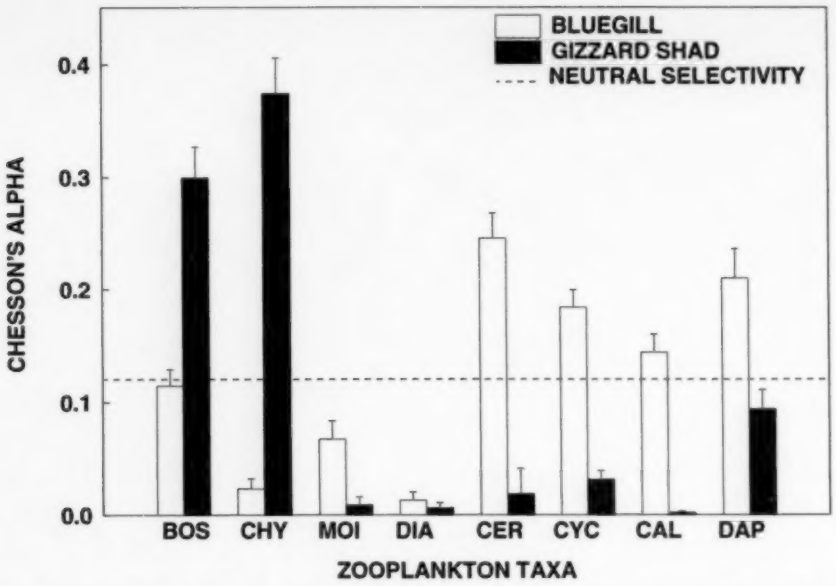

FIG. 2. Taxonomic selection (using Chesson's alpha, Chesson 1978, 1983) by larval bluegill and gizzard shad. Data represent averages across all containers ± 1 SE. The broken line represents neutral selection (i.e., 0.125, the reciprocal of the number of zooplankton taxa available). Zooplankton taxa: BOS = *Bosmina*, CHY = *Chydorus*, MOI = *Moina*, DIA = *Diaphanosoma*, CER = *Ceriodaphnia*, CYC = cyclopoid copepods, CAL = calanoid copepods, and DAP = *Daphnia*. Zooplankton taxa are placed in order of increasing size, from *Bosmina*, the smallest taxon, to *Daphnia*, the largest taxon.

ferences in gape between the two species were greatest for smallest larvae, declining with increasing total length.

Taxonomic Composition of Zooplankton

Because taxonomic composition varied across containers, we first evaluated the taxonomic composition of larval guts to determine if zooplankton taxa could be combined in our analysis of prey size. Neither bluegill nor gizzard shad guts were dominated by a single prey taxon. *Bosmina*, *Ceriodaphnia*, and calanoid and cyclopoid copepods were the most common prey of small bluegill. Larger bluegill tended to consume more calanoid copepods and *Daphnia*, but fewer *Ceriodaphnia* and *Bosmina*. For gizzard shad, *Bosmina* and *Chydorus*, followed by *Ceriodaphnia* and *Daphnia*, were the most common prey. Large gizzard shad tended to consume more *Chydorus* and fewer *Ceriodaphnia*. Overall, bluegill and gizzard shad diets varied most in the relative contribution of copepods and *Chydorus*, with copepods contributing substantially to bluegill gut zooplankton and *Chydorus* to gizzard shad gut zooplankton. This difference was mirrored in the average percent composition of zooplankton available to bluegill and gizzard shad. However, all of these taxa were available (although to varying degrees) to both bluegill and gizzard shad.

Accordingly, selectivity patterns varied between bluegill and gizzard shad (Fig. 2). Bluegill did not strongly select any particular taxon; in general, bluegill selected *Ceriodaphnia* and *Daphnia* and demonstrated slight selection for calanoid and cyclopoid copepods. Gizzard shad strongly selected *Chydorus* and *Bosmina*. Calanoid and cyclopoid copepods were avoided by gizzard shad.

Overall, bluegill and gizzard shad were exposed to, and consumed, a variety of prey taxa. Because our primary interest was the size of zooplankton eaten in relation to both larval gape and size of zooplankton available to larvae, zooplankton taxa were combined for statistical analyses. Below, when we state that a particular response variable increased or decreased or was related to a set of independent variables, these statements always will be significant at an alpha level of 0.05.

Prey Size Range across Containers

Across containers, substantial variation in prey size among larvae occurred (Fig. 3). For both bluegill and gizzard shad, minimum prey size remained fairly consistent among larvae across gape. However, maximum prey size appeared larger for bluegill than for gizzard shad. Further, more prey items exceeded gape for bluegill than for shad, probably because calanoid copepods, which are long but slender, were more common to bluegill guts than to gizzard shad guts. Our analyses were directed towards distinguishing the role of gape and available zooplankton size in driving these patterns of prey size of bluegill and gizzard shad.

Within-Species Foraging Patterns

To evaluate the contribution of gape and average available zooplankton size to larval prey size, we first regressed these two variables, as well as their interaction, against larval prey size (average prey size for each larval fish). Separate regressions were done for bluegill and gizzard shad. For both species, the model explained significant amounts of variability in larval prey size. However, the interaction

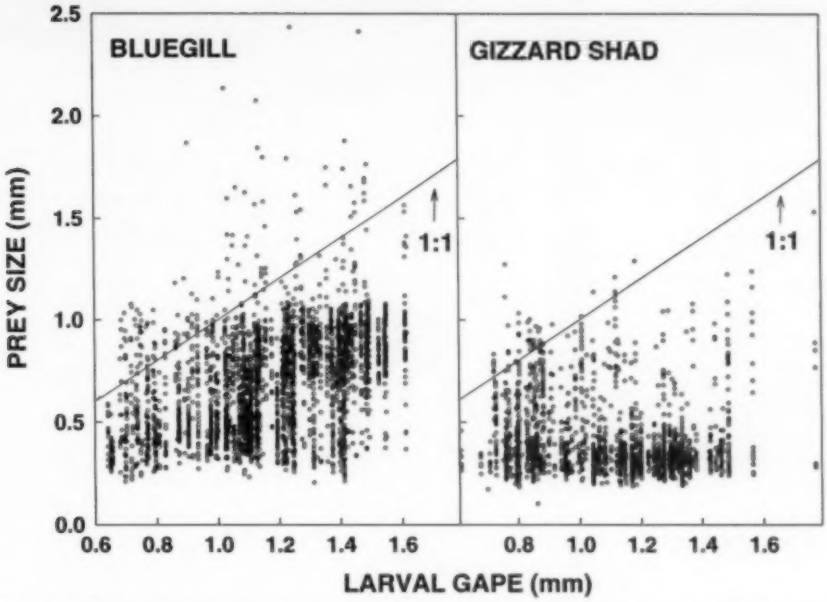

FIG. 3. Relationship between prey size and gape size for larval bluegill (n = 135) and gizzard shad (n = 155). Each point represents a single prey item. The solid line represents the 1:1 line where prey size = gape size.

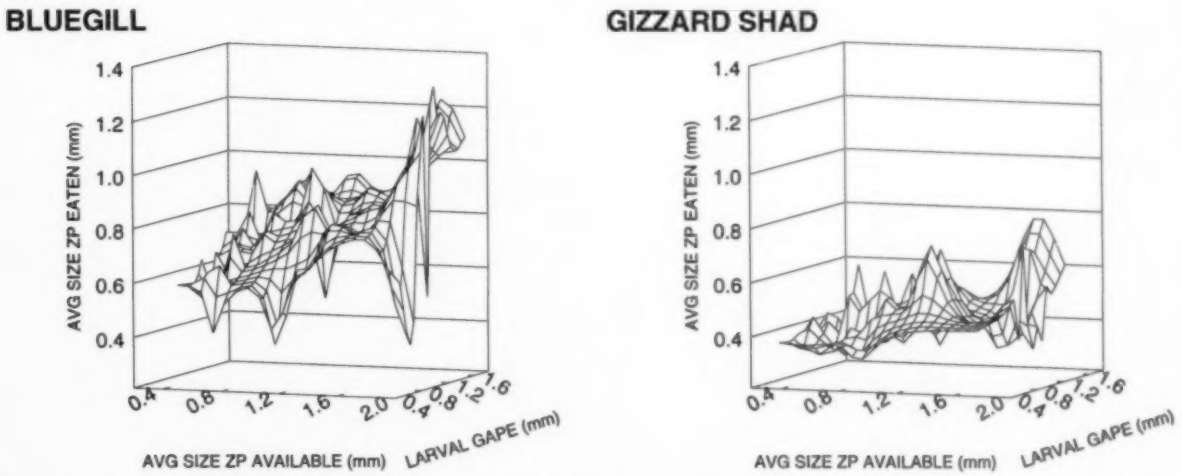

FIG. 4. Influence of increasing gape and average size of available zooplankton on average size of zooplankton eaten by larval bluegill (n = 135) and gizzard shad (n = 155). See Table 1 for regression statistics.

between gape and size of available zooplankton did not contribute to either model. Therefore, this term was dropped from further analyses, and we evaluated average prey size as a function of available zooplankton size and gape (no interaction term).

Bluegill prey size was explained by this model (Table 1). Specifically, prey size increased as both gape and available zooplankton size increased (Fig. 4). The gizzard shad model also revealed significant differences in prey size among individual larvae (Table 1). Specifically, gizzard shad prey size increased with available zooplankton size, but was unrelated to gape (Fig. 4). However, far less of the variation in prey size was explained by the gizzard shad model (r^2 = 0.12) than the bluegill model (r^2 = 0.54). In particular, the lower r^2 of the gizzard shad model indicates that variability in gizzard shad prey size was inadequately explained by gape and available zooplankton size. Further, a qualitative comparison of the equation coefficients reveals that prey size increased much more rapidly with available zooplankton size for bluegill than for gizzard shad.

These analyses reveal two important features of larval foraging. First, prey size for both bluegill and gizzard shad varied across experiments, indicating that larval prey choice varied across containers with different zooplankton assem-

TABLE 1. Regressions of average size of zooplankton eaten by larvae, calculated separately for bluegill (n = 135, r^2 0.54) and gizzard shad (n = 155, r^2 = 0.12), as a function of average size of available zooplankton and of larval gape (mm). The F statistic and p value are given for each full model, as well as the coefficients and associated standard error for individual model parameters. The p value for model parameters corresponds to the contribution of each parameter to the model, i.e., t-test for coefficient = 0.

	Bluegill			Gizzard shad		
Model:	F statistic = 77.25		p = 0.0001	F statistic = 10.83		p = 0.0001
Parameter:	Coefficient	SE	p	Coefficient	SE	p
Intercept	0.119	0.059	0.0414	0.344	0.047	0.0001
Zooplankton size	0.254	0.028	0.0001	0.132	0.028	0.0001
Gape size	0.351	0.051	0.0001	(−0.034)	0.044	0.446

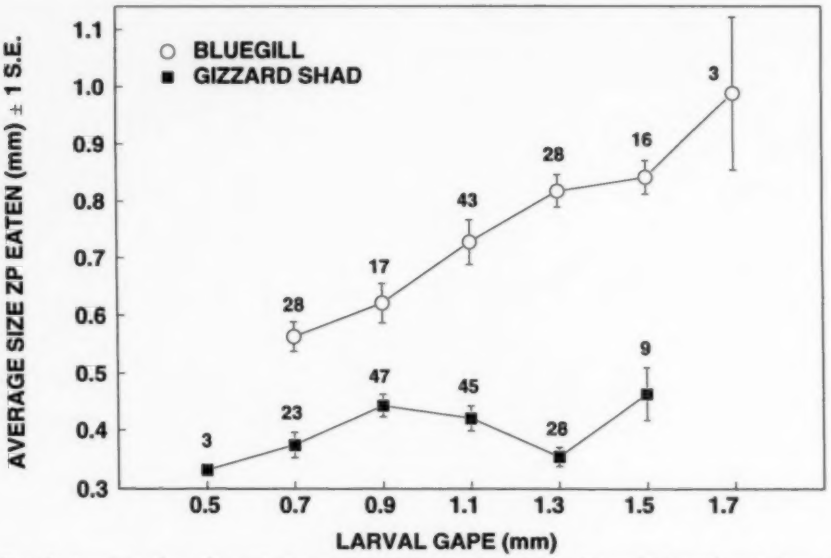

FIG. 5. Average size of zooplankton eaten by each size class of larval bluegill and gizzard shad. Data represent averages across all containers ± 1 SE. Sample sizes are presented above each data point.

blages or among larvae with different gapes. Second, differences in prey size were related to gape of bluegill, but not to gape of gizzard shad, suggesting that the relationship between gape and prey size varies across fish species.

Between-Species Comparisons

To compare prey size of larval bluegill and gizzard shad, we divided larvae into gape classes which spanned only 0.20 mm to minimize differences in gape among larvae within a size class, relative to the size range of zooplankton encountered. We calculated average size of zooplankton eaten by larvae in each gape class (across all containers), for each species, to determine if larvae with similar gapes ate similar-sized prey. Size of zooplankton eaten was influenced by both fish species (ANOVA: $F_{1,12} = 68.34$, $p = 0.0001$) and gape class ($F_{6,12} = 6.88$, $p = 0.0001$), as well as their interaction ($F_{5,12} = 6.11$, $p = 0.0001$; Fig. 5). Bluegill consumed larger prey than gizzard shad of the same gape, a difference that increased with gape.

Our previous analysis of each species indicated that bluegill and gizzard shad differed in response to available zooplankton size. To evaluate this pattern further, we regressed average prey size of all larvae against fish species, average size of available zooplankton, and their interaction (Table 2). Not surprisingly, average prey size was greater for bluegill than for gizzard shad and increased with size of available zooplankton. Further, the increase in prey size as available zooplankton size increased was greater for bluegill than for gizzard shad, indicating that the response to available zooplankton size varied between fish species.

To further characterize prey choice across zooplankton assemblages, we calculated size-selectivity values for each gape class of larval bluegill and gizzard shad. Because size-selectivity values incorporate sizes of available zooplankton, we could confirm that differences between bluegill and gizzard shad prey size did not derive from differences in the size distribution of zooplankton assemblages available to the two species. This analysis also allowed us to explore whether increases in bluegill prey size were due solely to opportunistic feeding (prey size increases with available zooplankton size) or due to selective feeding on larger zooplankton. In most cases, larvae consumed less than 25% of available zooplankton; only in 10 of 62 containers did larvae consume ≥25% of available zooplankton. Size-selectivity patterns were similar in analyses with and without these 10 containers, indicating that prey size selection was robust

TABLE 2. Regression of the average size of zooplankton eaten, including all larvae (n = 290, r^2 = 0.60), as a function of fish species, average size of available zooplankton, and the interaction between these two variables. In this analysis, bluegill were designated as fish species = 1 and gizzard shad as fish species = 2 (therefore, the negative coefficients for fish species and fish species × available zooplankton size indicate that bluegill ate larger prey than gizzard shad and that these differences in prey size were more marked when large zooplankton were available). The F statistic and p value are given for the full model, as well as the coefficients and associated standard error for individual model parameters. The p value for model parameters corresponds to the contribution of each parameter to the model, i.e., t-test for coefficient = 0.

Model:	F statistic = 144.91		p = 0.0001
Parameter:	Coefficient	SE	p
Intercept	0.639	0.061	0.0001
Fish species	−0.163	0.039	0.0001
Zooplankton size	0.447	0.065	0.0001
Fish species × zooplankton size	−0.160	0.043	0.0002

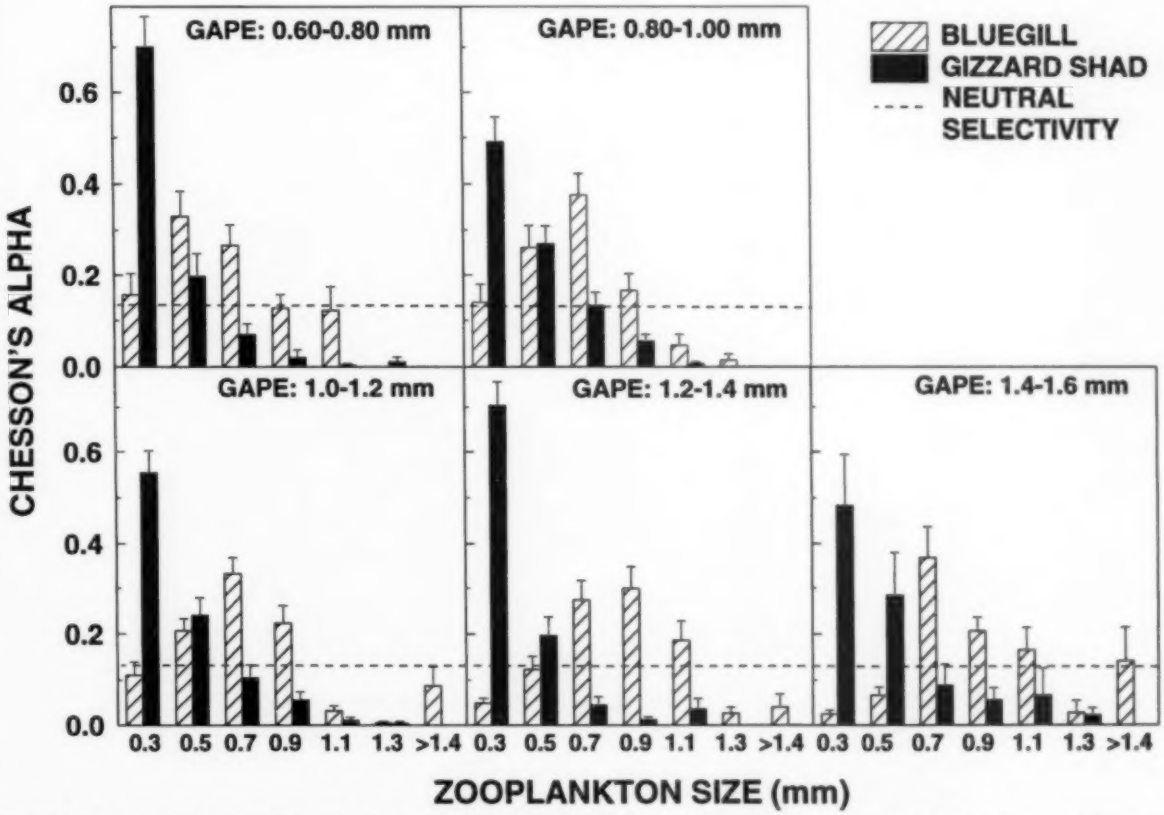

FIG. 6. Size selection (using Chesson's alpha, Chesson 1978, 1983) by larval bluegill and gizzard shad of each gape class. Data represent averages across all containers ± 1 SE. The broken line represents neutral selection (i.e., 0.14, the reciprocal of the number of zooplankton size classes available).

across zooplankton densities. Thus, we included all containers in our size-selectivity analysis.

To calculate selectivity, we partitioned gut and available zooplankton into seven size classes, representing 0.2-mm increments. We then calculated selectivity values for individual fish (for each zooplankton size class) and generated an average value for each larval size class. Bluegill consistently selected larger prey than similar-sized gizzard shad; further, bluegill selected larger zooplankton as gape increased whereas gizzard shad consistently selected the smallest zooplankton (Fig. 6).

Weight-specific Consumption

We also calculated the biomass of zooplankton consumed by larvae in each gape class to determine if consumption varied across zooplankton assemblages. To standardize con-

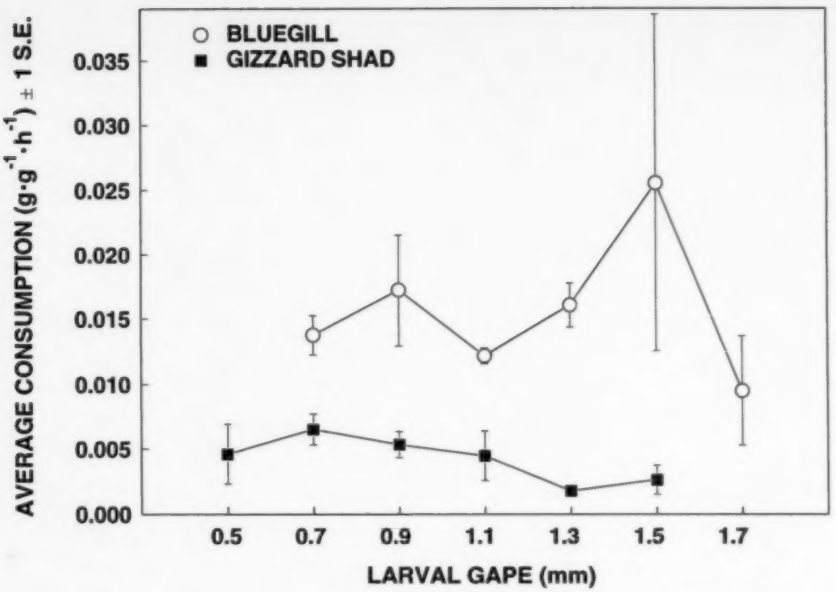

FIG. 7. Average weight-specific consumption (g zooplankton·g^{-1}·h^{-1} ± 1 SE) eaten by each size class of larval bluegill and gizzard shad.

sumption estimates across fishes, we generated estimates of grams of food per gram of fish per hour for each fish. Overall, bluegill consumption exceeded that of gizzard shad (ANOVA, fish species effect: $F_{1,12} = 13.63$, $p = 0.0003$) (Fig. 7). Weight-specific consumption was consistent across size classes for both species (size class effect: $F_{6,12} = 0.67$, $p = 0.677$; interaction effect: $F_{5,12} = 1.21$, $p = 0.305$).

To determine if consumption was related to gape size or size of available zooplankton, we regressed consumption (grams per gram per hour) against the average size of available zooplankton, larval gape, and the interaction between gape and size of available zooplankton for all bluegill or gizzard shad. Consumption by both species was unrelated to these variables (bluegill: F statistic = 1.31, $p = 0.28$, $r^2 = 0.03$; gizzard shad: F statistic = 0.77, $p = 0.51$, $r^2 = 0.01$).

Unlike the patterns in prey size, weight-specific consumption by bluegill and gizzard shad was unrelated to available zooplankton size. Bluegill consumed a larger number of small prey in small zooplankton containers relative to large zooplankton containers. (Bluegill also selected larger zooplankton across containers). As a result, consumption was equal across zooplankton assemblages. Because gizzard shad typically consumed few small prey, their consumption was low and consistent across experiments as well.

Discussion

A Multispecies Approach to Recruitment: the Role of Larval Gape

Overall, gape provided insight into size selectivity. Bluegill, the large-gaped species, fed on larger prey than gizzard shad, the small-gaped species. However, gape – prey size relationships differed markedly between bluegill and gizzard shad. Larvae of these species with the same gape selected different prey sizes; selectivity patterns with increasing gape differed as well. Both bluegill and gizzard shad fed on prey smaller than their gape, as in previous studies (Arthur 1976; Hansen and Wahl 1981; Lemly and Dimmick 1982; Michaletz et al. 1987), but this trend was more striking for gizzard shad than for bluegill. Size of zooplankton eaten would likely fall below gape because our measure of zooplankton size did not include morphological features such as spines. However, the strong selectivity for small zooplankton by gizzard shad (<0.50 mm) clearly indicates that gizzard shad fed on zooplankton much smaller than the maximum size their gape would allow. Therefore, whereas gape provided an indication of relative prey size, it alone did not accurately predict prey size for these two fishes, indicating that other factors influence larval foraging.

Whereas gape limits the size of zooplankton that can be consumed, visual acuity or foraging mode may determine which prey are detected and captured by larval fish (Hunter 1981; Hairston et al. 1982). As visual particulate feeders, larval bluegill (as well as adults) likely select large prey (within gape size constraints). Larval gizzard shad, typical of clupeid larvae, use a characteristic S-posture to capture prey, a mode of foraging unlikely to obtain larger, more evasive prey (Hunter 1981). Further, gizzard shad switch from this form of particulate feeding to filter-feeding at about 25 mm (Cramer and Marzolf 1970; Guest et al. 1990). As filter-feeders, gizzard shad are not size-selective predators; rather, they consume zooplankton with little ability to escape (Drenner et al. 1982). Gizzard shad >25 mm in our experiments may have been filter-feeding, thus capturing smaller, more vulnerable prey items rather than the larger, more evasive ones. Thus, differences in foraging behavior between bluegill and gizzard shad may underlie their different gape – prey size relationships, illustrating that studies of morphological features, such as gape, must recognize that behavioral differences may modify the role of any morphological feature, complicating direct species comparisons.

Because rotifers and copepod nauplii were not included in our design, we likely overestimated gizzard shad prey size because these small prey often occur in diets of young-of-year gizzard shad (Dettmers and Stein 1992; DeVries and Stein 1992). Hence, the disparity between bluegill and gizzard shad prey choice could be even greater. Further, gizzard shad and bluegill gapes differ most at smallest sizes; therefore, larvae smaller than those used may be even more vulnerable to the differential influence of zooplankton size and to food limitation in general. In particular, small gizzard shad larvae may require high densities of small zooplankton, such as rotifers and copepod nauplii, whereas bluegill may be able to consume larger zooplankton much earlier in their ontogeny.

Zooplankton Size

Implications for growth and survival

Given differences in prey choice between bluegill and gizzard shad, we hypothesized that differences in foraging success, resulting from differences in size of available zooplankton, would lead to differences in growth. To explore this issue, we calculated larval consumption during the foraging trials. While total weight-specific consumption did not vary in response to changes in available zooplankton size, as we would have predicted, this analysis is inconclusive because of the short-term nature of these experiments. Without estimates of foraging costs, such as search, capture, and assimilation, optimal zooplankton size for larval growth cannot be estimated. To quantify how zooplankton size influences larval fish growth, longer experiments in which growth is quantified are required. Growth differences resulting from zooplankton size could occur, even if prey biomass consumed is the same. Bluegill may spend more energy capturing the same overall biomass of prey when only small zooplankton are available as compared with when large zooplankton are available as well. Conversely, gizzard shad may invest more energy in search of a relatively low number of small zooplankton when the zooplankton assemblage is dominated by large individuals. Unfortunately, studies that have documented differences in young-of-year fish growth on different sizes of zooplankton have not measured larval consumption, making direct comparisons with our study difficult.

High zooplankton densities used in these experiments may have masked differences in consumption that could occur at low zooplankton densities. For example, when testing how zooplankton size influences growth of young-of-year yellow perch (*Perca flavescens*), Mills et al. (1989a) found that available zooplankton size was related to yellow perch growth when yellow perch were fed a 25% dry weight ration, but not when fed a 40% ration. Clearly, the energetics of foraging are likely to be influenced by both size and number of available zooplankton. High densities of zooplankton may alleviate, to some extent, the constraints that zooplankton size exerts on larval foraging.

Community implications

We contend that zooplankton size likely differentially affects growth and survival of larval fishes, particularly bluegill and gizzard shad. Specifically, systems containing large zooplankton may benefit young-of-year bluegill. Conversely, systems dominated by small zooplankton appear ideal for larval gizzard shad. Granted, systems dominated by large zooplankton will always contain some relatively small zooplankton in the form of early life stages of large zooplankton taxa. However, in systems dominated by large zooplankton taxa, both the relative and absolute abundance of these small zooplankton size classes will be lower than in a system (with similar total zooplankton densities) dominated by small zooplankton taxa. Therefore, encounter rates of larvae with these small zooplankton would be substantially lower (and thus foraging costs likely higher) in a system dominated by large zooplankton taxa.

Clearly, size structure of zooplankton assemblages varies considerably across systems (Brooks and Dodson 1965; Gannon and Stemberger 1978; Sprules 1980; Pace and Orcutt 1981; Bays and Crisman 1983; Pace 1986; Mills et al. 1987; Hall and Ehlinger 1989). In particular, many north temperate lakes are dominated by large zooplankton taxa (Brooks and Dodson 1965; Mills et al. 1987; Hall and Ehlinger 1989), in contrast with many Ohio reservoirs, in which small zooplankton taxa are most abundant (average size of zooplankton 0.3–0.5 mm; Bremigan et al. 1991; DeVries and Stein 1992). In turn, differences in the relative abundance of bluegill and gizzard shad between these two systems are evident as well. Ohio reservoirs typically support successful recruitment, and ultimately dense adult populations, of gizzard shad, which often vastly outnumber other fishes, such as bluegill (Johnson et al. 1986). While bluegill persist in Ohio reservoirs, they often do not reach densities typical of many north temperate lakes (i.e., in areas such as Wisconsin, Michigan, and New York). Gizzard shad, on the other hand, rarely reach high density in north temperate systems. However, the geographic distribution of gizzard shad extends north of the forty-fifth parallel (Becker 1983), indicating that temperature alone does not preclude them from all northern systems.

Throughout the foraging experiments, gizzard shad consistently selected and consumed small zooplankton, characteristic of many Ohio reservoirs. Conversely, bluegill consistently selected larger zooplankton that often are quite rare in Ohio reservoirs. Based on results of the foraging experiments, combined with field patterns in the distribution of these species, we hypothesize that differences in the size structure of zooplankton communities across systems could underlie patterns in species distributions. In particular, Ohio reservoirs, and other systems dominated by small zooplankton, may represent ideal foraging environments for gizzard shad. Gizzard shad typically spawn before bluegill and may reduce zooplankton abundances (Dettmers and Stein 1992; DeVries and Stein 1992). Therefore, bluegill may be disadvantaged in Ohio reservoirs due to both small zooplankton size and competition with gizzard shad for these less than ideal resources. Conversely, north temperate lakes dominated by larger zooplankton may represent ideal foraging environments for larval bluegill, but may constrain foraging success and ultimately recruitment of gizzard shad.

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Long-term Invertebrate Community Response to Toxaphene Treatment in Two Lakes: 50-yr Records Reconstructed from Lake Sediments

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Miskimmin, B.M., and D.W. Schindler. 1994. Long-term invertebrate community response to toxaphene treatment in two lakes: 50-yr records reconstructed from lake sediments. Can. J. Fish. Aquat. Sci. 51: 923–932.

Analysis of sediment cores from two toxaphene-treated and one untreated lake basin demonstrates both the short-term effect of toxaphene applied in 1961–62 and the longer term effect of subsequent trout stocking on invertebrates. In Chatwin Lake (higher toxaphene concentration), planktonic cladocerans decreased in abundance, and dominance quickly changed from small- to large-bodied types. *Bosmina* was reduced by 88% at the time of toxaphene application and was eliminated during the 1970's as invertebrate predators like *Chaoborus americanus* increased in response to the poor survival of stocked fish. Short-term toxic effects were not detectable in cores from Peanut Lake (lower toxaphene concentration). In both treated lakes, large invertebrates became dominant with the absence of native fish and during trout stocking. *Chaoborus* spp. remained at low levels throughout the 1960's in both treated lakes compared with the untreated lake, possibly due to residual toxaphene toxicity and/or to predation by stocked fish. Toxicity to total chironomids was not detected, although genera-specific responses were not analysed. While the higher toxaphene dosage caused residual toxicity in Chatwin Lake for at least a decade, the manipulation of fish communities was primarily responsible for long-term changes in the invertebrates of both lakes.

L'analyse de carottes sédimentaires provenant de deux bassins de lac traités au toxaphène et d'un bassin non traité, montre à la fois les effets à court terme du toxaphène appliqué en 1961–1962 et l'effet à long terme sur les invertébrés de l'ensemencement subséquent par des truites. Dans le lac Chatwin (concentration supérieure en toxaphène), les cladocères planctoniques ont diminué en abondance et la dominance est rapidement passée des sujets de petite taille aux sujets de grande taille. La population de *Bosmina* a été réduite de 88 % au moment de l'application du toxaphène; elle a été éliminée au cours des années 1970 alors que des prédateurs invertébrés comme *Chaoborus americanus* sont devenus plus abondants, étant donné la mauvaise survie du poisson introduit dans le lac. Les effets toxiques à court terme n'étaient pas détectables dans les carottes du lac Peanut (concentration inférieure en toxaphène). Dans les deux lacs traités, les gros invertébrés sont devenus dominants en l'absence des espèces indigènes de poissons et durant la période d'ensemencement avec la truite. *Chaoborus* spp. est resté peu abondant tout au cours des années 1960 dans les deux lacs traités à comparer au lac non traité; cela est peut-être attribuable à une toxicité résiduelle du toxaphène et (ou) à la prédation exercée par le poisson introduit. On n'a pas détecté de toxicité pour l'ensemble des chironomidés; cependant, les réponses spécifiques aux genres n'ont pas été analysées. Bien que la concentration supérieure de toxaphène ait exercé une toxicité résiduelle dans le lac Chatwin pendant au moins une décennie, la manipulation des communautés de poissons a été le principal facteur des changements à long terme dans la composition en invertébrés des deux lacs.

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Toxaphene is a mixture of polychlorinated camphenes (PCC) that was once widely used as a piscicide and an agricultural pesticide in both Canada and the United States. Now banned for most uses in North America, toxaphene is still used in other parts of the world. Toxaphene and its residues are among the persistent organochlorines that are considered global pollutants because they are carried in the atmosphere and are found in ecosystems far from the original sites of utilization (Rice and Evans 1984; Muir et al. 1988; Van der valk and Wester 1991). During the 1950's and 1960's, numerous provincial and state agencies considered toxaphene a less expensive and more lethal alternative to rotenone for extirpating undesirable fish from lakes prior to stocking with various species of trout ("lake rehabilitation", Cumming 1973).

Toxaphene application to lakes has caused both direct and indirect ecosystem damage. Monitoring studies revealed poor survival of stocked trout for many years (Terriere et al. 1966; Webb 1980), bioaccumulation by aquatic plants and animals (Terriere et al. 1966), and detrimental effects on nontarget organisms in the lakes (e.g., chironomids, Cushing and Olive 1957; cladocerans, amphipods, Larkin et al. 1970; three species of *Chaoborus*, Hilsenhoff 1971; *Chaoborus punctipennis*, Webb 1980). The time for recovery of invertebrates following toxaphene application varied from many months (Cushing and Olive 1957; Hooper 1959) to several

TABLE 1. Characteristics of the study lakes treated with toxaphene in 1961–62.

	Chatwin Lake	Peanut Lake
Latitude	54°15′N	54°01′N
Longitude	110°51′W	114°21′W
A_0	71 ha	24 ha (treated), 5 ha (untreated)
Z_{max}	15.8 m	14 m[a]
pH	9.2	9.1
Conductivity	1400 $\mu S \cdot cm^{-1}$	510 $\mu S \cdot cm^{-1}$
Alkalinity	16.4 $meq \cdot L^{-1}$	6.2 $meq \cdot L^{-1}$
TP	47 $\mu g \cdot L^{-1}$	20 $\mu g \cdot L^{-1}$
TDP	37 $\mu g \cdot L^{-1}$	9 $\mu g \cdot L^{-1}$

[a]Both basins have similar depths; other characteristics are approximate ice-free season values of the treated basin.

years (Larkin et al. 1970; Hilsenhoff 1971; Webb 1980). Unfortunately, monitoring programs were relatively scarce, and either too brief or not comprehensive enough to evaluate the longer term effects on lacustrine communities.

In addition to the effects of toxaphene, the replacement of a native and diverse fish community by a single salmonid population would be expected to modify the invertebrate communities in lakes. Any significant change in fish density and/or feeding habits could alter the types, sizes, and abundances of food-base organisms (Anderson 1970; Carpenter et al. 1985; Leavitt et al. 1989). With few vertebrate predators (planktivores) present, large invertebrate predators and large herbivores (like some daphnids) usually increase causing reductions in small crustaceans (Brooks and Dodson 1965; Shapiro and Wright 1984). Further, when lakes have few or no planktivorous fish, *Chaoborus americanus* often becomes the dominant chaoborid because it does not migrate downward into darker waters during the day to avoid predation by fish (Borkent 1981) and because it can outcompete smaller chaoborids (Pope et al. 1973; Von Ende 1979; Uutala 1990). Even assuming that trout fry diets were similar to some native planktivores (Scott and Crossman 1973), predation pressure on invertebrate prey would be constrained by the absence of natural recruitment of fish and dependence on the stocking success of hatchery trout.

Close-interval sampling of lake sediment cores can provide long-term records of many organisms residing in and around lakes (Frey 1988). Lake sediments sometimes accumulate in undisturbed laminae that permit accurate temporal analyses of invertebrate and plant communities. An example of the utilization of chaoborid and chironomid remains from sediment cores was the reconstruction of the historical status of fish populations (acidified lakes in Ontario, Johnson et al. 1990), one of the aims of our study. We quantify chironomids, chaoborids, and cladocerans which have species-specific body parts that preserve well in sediments. When the chronology of sediment deposition can be established, these members of the aquatic community can be enumerated and can supplement existing limnological data.

Here we examine the response to toxaphene application and stocking with a nonnative fish species on total chironomids, *Chaoborus* spp., and planktonic Cladocera in a mesotrophic and a eutrophic lake in central Alberta, Canada. We compare the response in the lake that received a low toxaphene dose with one with a higher dose and contrast the results with a lake basin that was not treated. Our objectives were to establish a long-term record of some members of the invertebrate community prior to 1961–62 toxaphene applications, determine any patterns in toxic response, and examine the recovery of the communities in the 30 yr following disturbance.

Materials and Methods

Study Sites

The central Alberta lakes were chosen for coring because of their common history of toxaphene treatment and trout stocking, their suitable morphometry for accumulating laminated sediments (small, deep seepage lakes), and differences in productivity and chemical characteristics (Table 1). Both lakes are in the Boreal Mixedwood Ecoregion (*Populus tremuloides* dominated). The lakes have a sparsely documented chemical and fisheries history, most of which was gathered by Alberta Department of Fish and Wildlife, and is available to researchers (referred to hereafter as AFW files)

Eutrophic Chatwin Lake (Table 1) has two cattle farms (established prior to 1952 and expanded in the 1980's) and a golf course in its otherwise forested watershed. Prior to toxaphene treatment (0.0184 ppm) in 1962, the fish community consisted of northern pike (*Esox lucius*), yellow perch (*Perca flavescens*), and brook stickleback (*Culaea inconstans*) (AFW files; Fig. 1). Chatwin Lake was stocked in 14 of 28 yr between 1964 and 1992 with a total of 980 000 hatchery rainbow trout (*Oncorhynchus mykiss*) (mean of 1100 fish·ha^{-1} per stocking event). Survival of stocked trout has been very poor, and other fish species are absent (AFW files).

Chatwin Lake had clearly laminated sediments in all cores that were collected. At present, this lake has a high density of littoral and pelagic Amphipoda (*Gammarus lacustris*, *Hyallela azteca*). The order of numerical abundance of zooplanktonic organisms sampled in midsummer 1992 with a 64-μm-mesh plankton net was *Asplanchna* (Rotifera, Ploima) > *Daphnia pulex* (Cladocera) = *Acanthodiaptomus denticornis* (Calanoida) > *Chaoborus flavicans* (Diptera) > *Keratella* (Rotifera, Ploima) > *C. americanus* >> *Ceriodaphnia* sp. (Cladocera).

Mesotrophic Peanut Lake has a cattle farm (established 1911) in the eastern portion of its watershed but is otherwise relatively undeveloped. The lake comprises two basins that were joined prior to toxaphene treatment, but permanently divided in the same year as toxaphene treatment by construction of an earth berm across the narrows (Fig. 1). Prior to treatment, the lake contained walleye (*Stizostedion vitreum*), yellow perch, and various small cyprinids (AFW files). Some walleye and yellow perch were tranferred from a nearby lake to Peanut Lake in the 1930's (AFW files), although a clear record of whether either of these taxa was present prior to this does not exist. Toxaphene (0.0075 ppm) was applied to remove all fish from the north basin in 1961, leaving the south basin untreated.

The treated basin of Peanut Lake was first stocked in August 1965 and has been stocked annually since 1967 with hatchery rainbow trout (total of 330 000 fish; mean of 600 fish·ha^{-1} per stocking event). It also currently supports fathead minnow (*Pimephales promelas*), brook stickleback, and a few walleye (a single 1-kg specimen was caught by the authors in 1991). These species may have been reintroduced

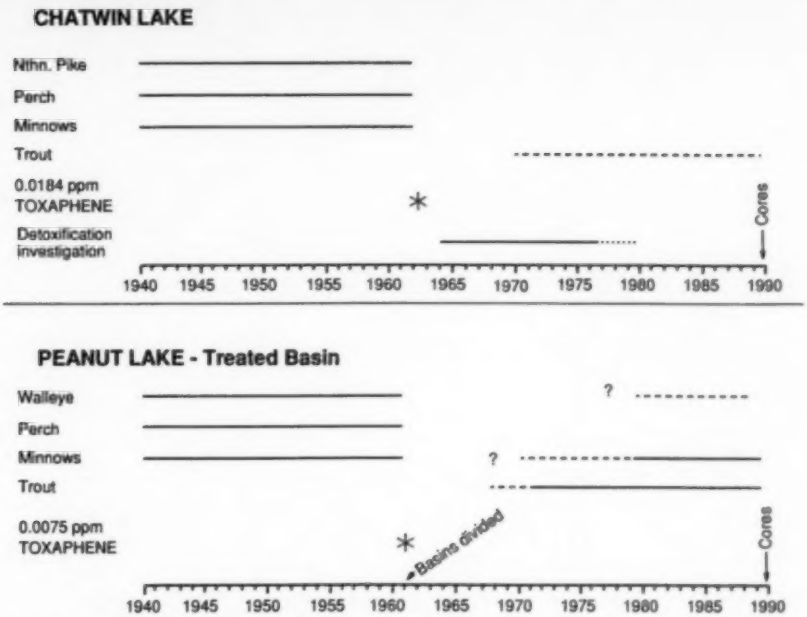

FIG. 1. Chronological history of known events in Chatwin and Peanut lakes, Alberta, from 1940 to 1990.

by anglers (D. Berry, Alberta Fish and Wildlife, 5th floor, 9920–108 St., Edmonton, AB T5K 2M4, personal communication). No current records for fish in the untreated basin of Peanut Lake exist. The untreated basin was thought to maintain a yellow perch population in the 1950's and early 1960's, prior to and shortly after the basins were divided (AFW files). Fish are probably depleted or absent now in this smaller basin because oxygen is occasionally reduced to extremely low levels (0–0.5 $mg \cdot L^{-1}$) in the winter under ice (AFW files).

The treated basin of Peanut Lake has laminated sediments with the exception of an 11.5-cm interval of unlaminated material (~9.5–21 cm depth, referred to hereafter as a "slump"), possibly from earth introduced into the lake during construction of the berm between the basins just after toxaphene treatment (Fig. 1; Don Hove, County of Barrhead Public Works Department, Barrhead, Alta., personal communication). Two other physical markers were useful in matching depths between core sections: 0.5- to 1-cm light-coloured bands at 24 and 30 cm. The untreated basin has laminated sediments that are uninterrupted by mixed intervals as occurred in the treated basin. The order of numerical abundance of zooplanktonic organisms sampled in midsummer 1992 in the treated basin with a 64-μm-mesh plankton net was small cyclopoids (*Diacyclops* sp.) = calanoids (*Hesperodiaptomus* sp.) > *Daphnia pulex* = *Daphnia rosea* > *Bosmina* (Cladocera) > rotifers.

Core Collection

Two or three sediment cores were collected by freeze-coring (O'Sullivan 1983) at the deepest site on each of the three lake basins (Chatwin, Peanut treated and untreated basins). Distinct laminations verified that sediments were relatively undisturbed by the coring process. The cores were wrapped in aluminum foil, returned to the laboratory on dry ice, and stored at −10°C for further use. Cores were quartered lengthwise with a band saw, the exterior cleaned with a woodplane to remove contaminated surfaces, and thawed or freeze-dried before sampling. Sections of cores were analysed for cladoceran and dipteran macrofossils, organochlorines, ^{210}Pb chronology, and organic content. Because all analyses could not be done on individual quarter sections, a total of four or five quarter sections for each basin were used to complete the study. Between-section depth correlation was achieved by matching analogous laminae or other markers such as the distinctive light-coloured bands.

Dating and Organic Content

A combination of varve estimates (Chatwin Lake), physical markers and ^{210}Pb chronology (all three basins), and toxaphene analyses (the two treated basins) were used for dating sediments. Varves (annual sets of laminae) in Chatwin Lake were distinguished as regularly repeated colour bands and were counted independently by two individuals. ^{210}Pb chronology was established by sampling 2–5 mL of sediments per sample from a thawed core and assaying by the constant rate of supply model described by Eakins and Morrison (1978).

Toxaphene was determined on freeze-dried 0.8- to 1.0-cm slices of a quarter section. The slices were made by band sawing a frozen section and were individually prepared (while frozen) by removing inner smeared surfaces and outer contaminated edges using a stainless steel knife. Analyses were completed on freeze-dried samples by selected-ion, electron capture detector gas chromatography (Varian 6000) as described by Muir et al. (1988). Toxaphene results were verified by negative chemical ionization mass spectrometry. Toxaphene maxima are used here only to confirm the year and depth of toxaphene application.

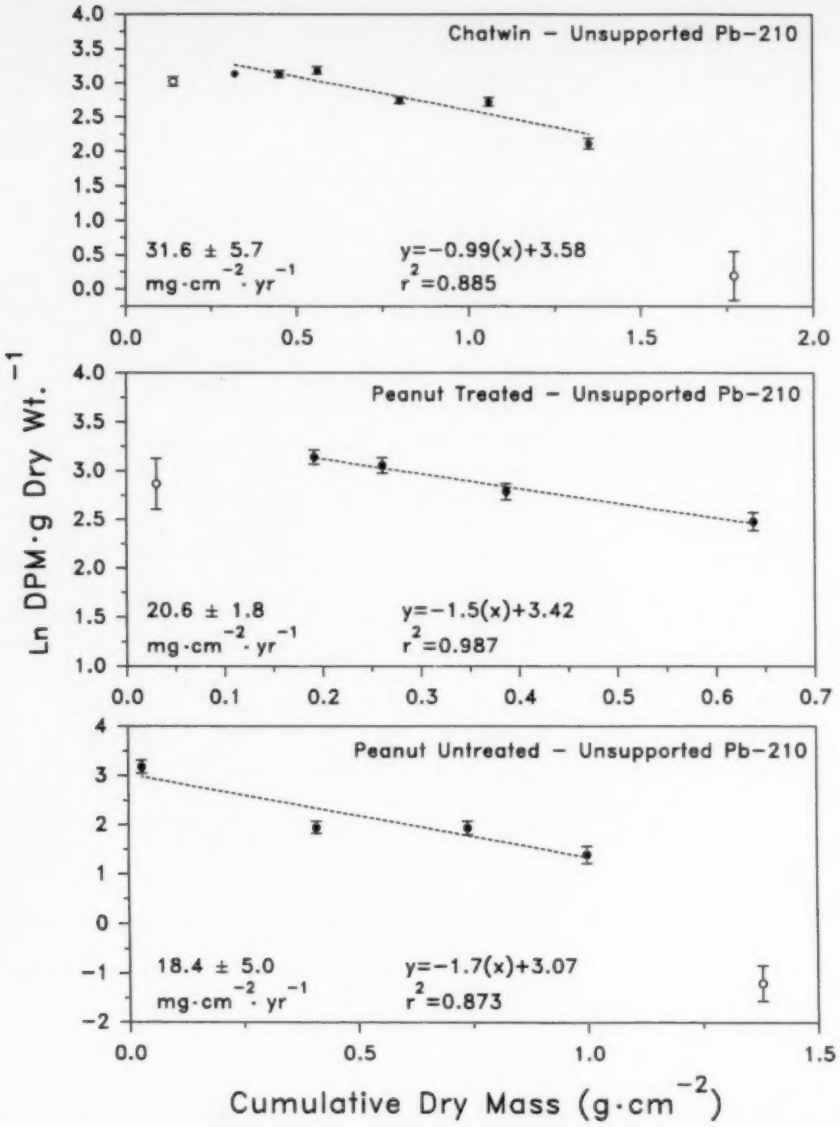

FIG. 2. Unsupported ^{210}Pb activity profiles from Chatwin Lake and from the treated and untreated basins of Peanut Lake. Mean accumulation rates shown were derived from a ratio of the ^{210}Pb decay constant (−0.03112) and slope of the lines. Empty points are not included in the equation because counts were too low or too close to background.

Organic content, defined as loss on ignition (LOI), was measured in core sections from the two treated lakes. Sediments were lyophylized (Virtis Freezemobile 6) for 24 h. This process left a 4-mm freeze-dried zone over a frozen base for the full length of the cores (~20–30 cm). Sections of the dried material at 0.5-cm intervals were subsampled and weighed. LOI was determined by weight difference before and after burning at 550°C for 1.5 h.

Cladocerans

Samples were taken at 0.5- to 1.0-cm intervals from a thawed core section. Following the general procedure of Frey (1986), 1 mL of wet sediments was added to 15 mL of 10% KOH, gently heated, and occasionally swirled for 5 h. This period was adequate to deflocculate most particles in samples from these lakes. Supernatant KOH was decanted and the sediments diluted with distilled water at least three times. The final volume was brought to 5 or 10 mL and subsampled to slides (50 μL per slide) using glycerin jelly stained with lignin pink as a mounting medium (Hann 1989). A Nikon phase contrast microscope was used to identify and enumerate *Daphnia* sp. and *Bosmina* remains (also Chydoridae; data not shown here) from four slides (200 μL) or until counts exceeded 150 per sample. Because *D. rosea* are found in recent plankton samples, but have postabdominal claws that are not unique, claws of the type

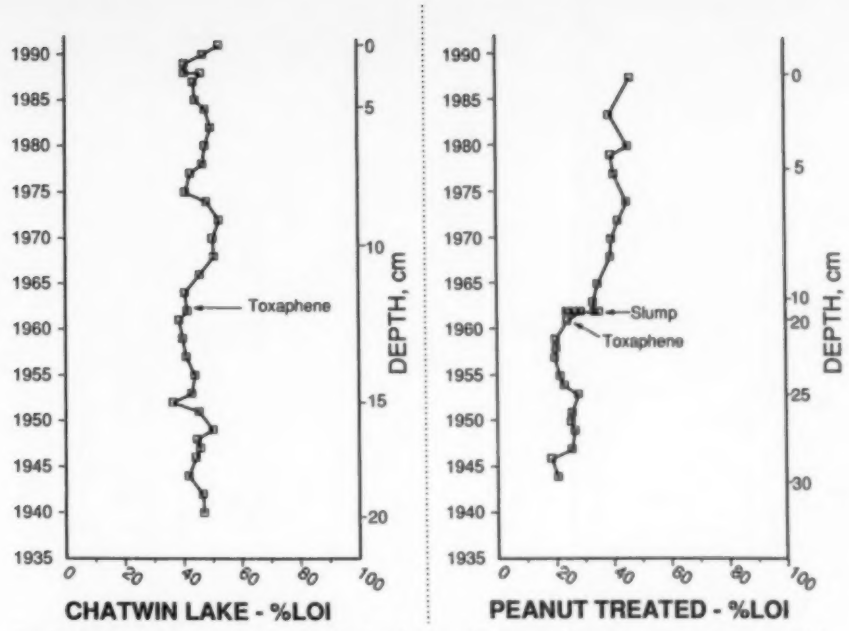

FIG. 3. Percent LOI at ~0.5-cm intervals for the length of cores from each toxaphene-treated lake. Left axes show dates; right axes are depths corresponding to the dates.

are reported as this species. Note that *D. rosea* are not distinct from *Daphnia galeata mendotae* based on claw morphology. *Daphnia galeata mendotae* were identified in archived samples from Peanut Lake collected in the early 1980's by University of Alberta staff. Data are reported as accumulation of pairs of *Daphnia* claws or *Bosmina* headshields representing individuals per square centimetre per year. Identification of remains was based on Brooks (1959), Pennak (1978) and Hann (1989).

Dipteran Macrofossils

Three-millilitre samples were taken from a thawed core section and deflocculated with 20–25 mL of 10% KOH similar to the cladoceran procedure. Invertebrate remains in the slurry were concentrated onto 93-μm mesh and rinsed into a plankton counting tray. No attempts were made to quantify any remains that passed through the mesh. These might include the unidentifiable earliest instars for this group. *Chaoborus* sp. mandibles and total chironomid head capsules were enumerated using a dissecting microscope. *Chaoborus* species were identified based on the mandible key of Uutala (1990). Results are reported as accumulation of mandibles (chaoborid) or head capsules (chironomid) per square centimetre per year.

Results

Dating

Accurate historical reconstruction of events in the two treated and one control lake basins was possible with the combined dating techniques of ^{210}Pb, toxaphene analyses, and varve counts (where possible). All sediment dating results are given on a dry weight basis.

The ^{210}Pb profile (Fig. 2), varve counts, and a toxaphene maximum (1600 ng·g^{-1}) in Chatwin Lake confirm the sediment depth of the 1962 treatment at 11.5 cm. Sediments have accumulated in this eutrophic lake at 31.6 ± 5.7 mg·cm^{-2}·yr^{-1} for the period of record and were well laminated throughout the length of the cores.

^{210}Pb profiles for Peanut Lake indicate an average sediment accumulation rate of 20.6 ± 1.8 mg·cm^{-2}·yr^{-1} for the treated basin (Fig. 2) and 18.4 ± 5.0 mg·cm^{-2}·yr^{-1} for the untreated basin (Fig. 2). Dating by varve counts in the treated basin cores was not possible because of the 11.5-cm slump (between 9.5 and 21 cm). The length of the slump was removed for the purposes of ^{210}Pb dating because it accumulated very quickly and independently of the true sediment accumulation rate in the lake. The depth of the toxaphene maximum of 500 ng·g^{-1} coincided with ^{210}Pb estimates of the 1961 treatment depth (below the slump) at approximately 21 cm. The same year is represented at approximately 9.3 cm in the untreated basin.

Organic Content

The organic content (defined by LOI) of sediments in Chatwin Lake averaged 45.1 ± 4.0% and in Peanut Lake (treated basin) averaged 29.8 ± 8.2% over the past 40–50 yr (Fig. 3). While sediment organic content in Chatwin Lake did not vary systematically, organic content in Peanut Lake increased substantially from the pre-toxaphene years (pre-1961: 23.7 ± 2.9%) to the post-slump years (post-1962: 39.8 ± 4.3%).

Invertebrate Communities prior to Toxaphene Treatment (1940–61/2)

Prior to toxaphene treatment, *Bosmina* and chironomids were the most abundant invertebrate remains found in Chatwin Lake sediments (Fig. 4a). During the 1940's and again in the late 1950's, large herbivores (*Daphnia* spp.) were relatively abundant, although *Bosmina* was the domi-

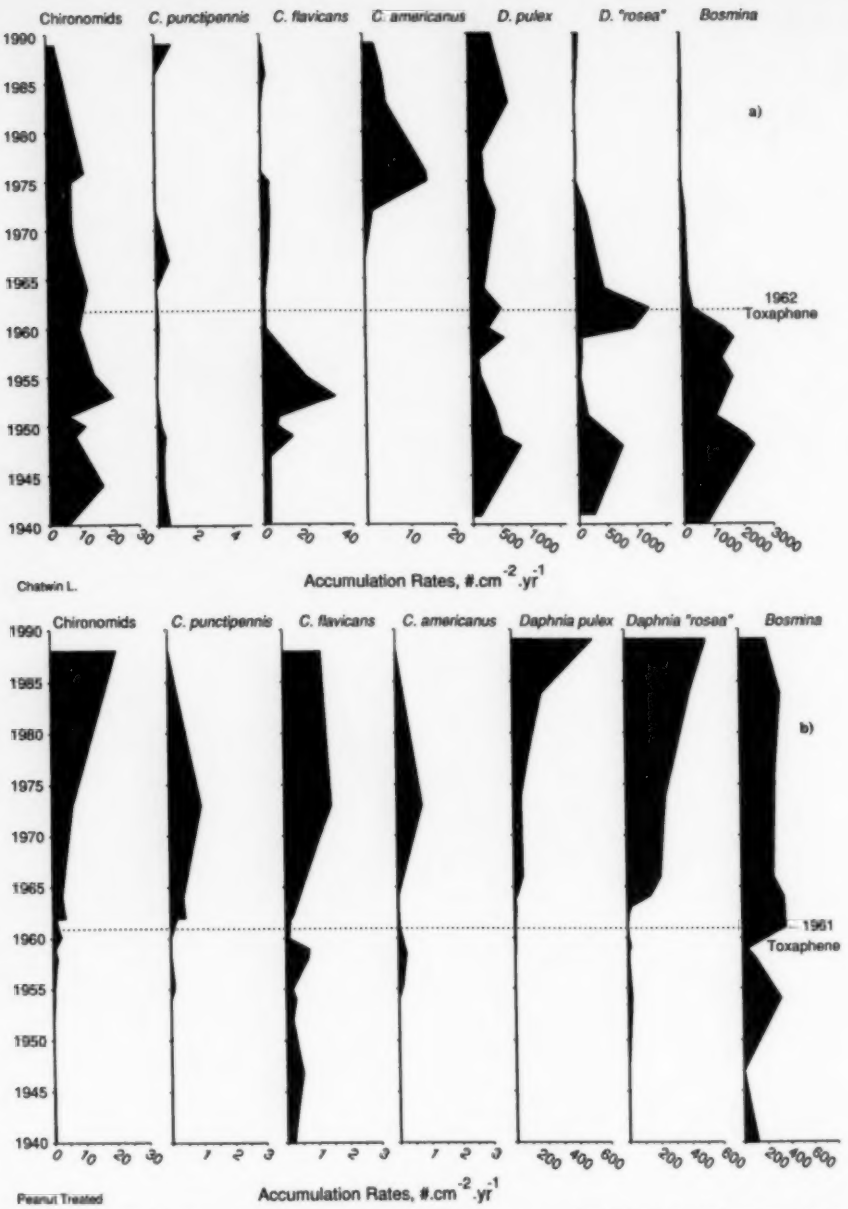

FIG. 4. Accumulation rates of chironomids, *Chaoborus* spp., and planktonic cladocerans from sediment cores over 50 yr in (a) Chatwin Lake, (b) Peanut Lake, treated, and (c) Peanut Lake, untreated. Chironomids = head capsules (total only), *Chaoborus* spp. = mandibles, *Daphnia* spp. = claw pairs ("*rosea*" in quotes due to similarity of claws to other types), *Bosmina* = headshields. (*Fig. 4 concluded next page*)

nant cladoceran in all pretreatment samples examined. Large numbers of *C. flavicans* were present in Chatwin Lake for a few years in the early to mid-1950's.

From 1940 through 1960, Peanut Lake supported low abundances of the invertebrates examined in this study (Fig. 4b). *Bosmina* was the dominant cladoceran in both the treated and untreated basins (the basins were not permanently divided during this period). *Chaoborus flavicans* was moderately abundant in both basins; however, chironomids accumulated at relatively higher rates in the smaller (untreated) basin (Fig. 4b and 4c).

Invertebrate Changes Immediately following Toxaphene Application (1961–64)

Permanent changes in the planktonic invertebrate community composition occurred in Chatwin but not in Peanut Lake following toxaphene application.

In Chatwin Lake, *Bosmina* was dominant prior to treat-

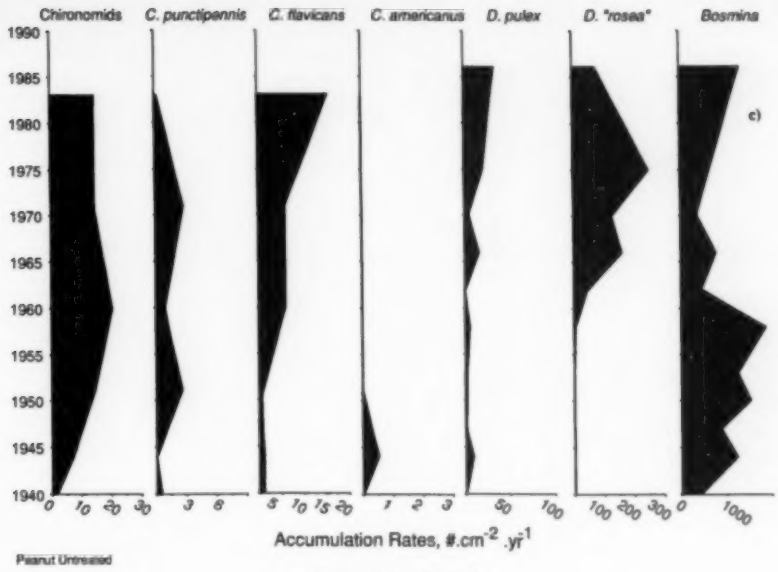

FIG. 4. (*Concluded*)

ment, but numbers were reduced by 88% at the time of toxaphene treatment (Fig. 4a). A short-term increase (1–2 yr) in *D. rosea* occurred after toxaphene treatment, while *D. pulex* was unchanged. No change in total chironomid accumulation was detected in sediments representing the year of toxaphene treatment. Chaoborids were found at low levels both shortly before and after treatment in Chatwin Lake.

In contrast, in Peanut Lake, *Bosmina* increased shortly (1–2 yr) after treatment. No such increase occurred at this time in the untreated basin, where instead, *D. rosea* commenced a steady increase in number (Fig. 4b and 4c). Because daphnids, chaoborids, and chironomids were present at low abundances in the treated basin of Peanut Lake prior to treatment (Fig. 4b), toxic effects were not detectable.

Post-toxaphene Changes (mid-1960's–1990)

Long-term changes in the invertebrates following manipulation of Chatwin and Peanut lakes exhibit some similarities, including changes in dominance from small cladocerans to large ones (*Bosmina* to *Daphnia*, and/or small *Daphnia* sp. to large) and increases in invertebrate predators like *Chaoborus* spp. (Fig. 4a and 4b). Significant increases in numbers of *Chaoborus* spp. did not occur until the late 1960's (Peanut, Fig. 4b) to early 1970's (Chatwin, Fig. 4a) despite the poor survival of trout throughout most of the 1960's.

In Chatwin Lake, an increase in *C. americanus* coincided with the elimination of *Bosmina* (already depleted by toxaphene). Chironomids and *D. pulex* were relatively stable since before the treatment, and *C. flavicans* never again reached the high numbers noted in the 1950's (Fig. 4a). In contrast, in the treated basin of Peanut Lake, all taxa considered have increased from the late 1960's onward to their highest levels in the 50-yr record (Fig. 4b). *Chaborus flavicans* became the dominant chaoborid and *Bosmina* was well represented in samples from Peanut Lake (unlike in Chatwin Lake). Note that most of the abrupt changes occurred after the slump of 1962 (year after toxaphene treatment) in the treated basin of Peanut Lake (Fig. 3 and 4b).

Daphnia rosea and *D. pulex* were rare in both the treated and untreated basins of Peanut Lake before treatment (and basin division). After 1961 (toxaphene treatment), increases in the small *D. rosea* preceded increases in the larger *D. pulex* by several years in both basins. Increases in both species of *Daphnia* and in *Chaoborus* occurred in the mid-1960's in both the treated and untreated basins. During the 1970's, small planktonic cladocerans, *Bosmina*, and *D. rosea* were more abundant than *D. pulex* in both basins. During the 1980's, *D. pulex* became co-dominant with *D. rosea* in the treated basin of Peanut Lake, but remained at lower levels than the smaller cladocerans in the untreated basin.

Discussion

Toxaphene Effects

The reported poor survival of stocked rainbow trout in both toxaphene-treated lakes for many years after treatment did not occur in rotenone-treated lakes in Alberta and initiated concerns about residual toxaphene toxicity to trout (AFW files). While it is not uncommon for small lakes in central Alberta to winterkill, oxygen depletion was not usual in the treated basins of Peanut and Chatwin lakes (AFW files), making residual toxaphene a conceivable problem. The failure of trout to survive in toxaphene-treated lakes in other areas was attributed to consumption of toxaphene-contaminated food organisms (Schoettger and Olive 1961; Johnson 1966). In addition to acute toxicity, toxaphene bioconcentrates by several orders of magnitude in food chains (e.g., 10^4 over water concentration for trout, Eisler and Jacknow 1985). Residual toxicity of toxaphene in treated lakes has not been reported to extend for more than a decade. Thus, while toxaphene might have caused residual toxicity problems in the 1960's, the reason for poor trout survival in the 1970's and beyond, despite intensive stocking in these prairie lakes, is unknown.

Stronger effects of toxaphene to invertebrates in Chatwin Lake than in Peanut Lake (Fig. 4a. and 4b) may be the result

of the higher concentration of toxaphene used in Chatwin Lake (Fig. 1). The concentration of toxaphene in Peanut Lake (0.0075 ppm) was one of the lowest levels recommended to achieve a complete fish kill (Hooper 1959), while the Chatwin Lake dose was three times higher.

Specifically, differences in *Bosmina* survival between lakes may have resulted from the higher toxaphene concentration used in Chatwin Lake. A recommended nonlethal concentration of 0.01 ppm toxaphene for *Bosmina* (Novak and Passino 1986) was exceeded in Chatwin but not in Peanut Lake. *Daphnia pulex* and *Daphnia magna* are less sensitive (nonlethal concentrations of 0.08 and 0.1 ppm) to toxaphene than *Bosmina* (Novak and Passino 1986), perhaps explaining daphnid survival in Chatwin Lake. Further, the International Joint Commission of the United States and Canada (1977) recommended a standard of 0.008 ppm toxaphene for protection of aquatic life. This standard was exceeded by severalfold in the case of Chatwin Lake and suggests that a lethal dose of toxaphene may have initiated the continuous decline of *Bosmina*.

In Chatwin Lake, it is possible that residual toxaphene was toxic for *Chaoborus* for many years. This lake sustained only low populations of *Chaoborus* spp. larvae throughout the 1960's despite the close proximity of other water bodies as colonization sources. In Chatwin Lake, no fish except low numbers of stocked trout have been recorded since toxaphene treatment, diminishing vertebrate predation as a reason for low *Chaoborus* populations. In a Wisconsin lake that was treated with toxaphene, *Chaoborus* spp. failed to recolonize until 4 yr later (Hilsenhoff 1971). In an Ontario study, where lake morphometry and treatment were very similar to Chatwin Lake (A_0 = 65 ha, Z_{max} = 16 m, toxaphene = 0.015 ppm), *C. punctipennis* shifted from representing 58% of benthic invertebrate abundance to complete extirpation at all depths following toxaphene treatment (Webb 1980). Webb also noted the poor survival of stocked trout (splake) and concluded that toxaphene (not predation) caused the complete absence of *Chaoborus* for the entire 4 yr of monitoring.

In Peanut Lake, it is impossible to distinguish the relative contributions of residual toxaphene and other factors for the poor recruitment of *Chaoborus* spp. through the decade after toxaphene treatment. The untreated basin is the only one of the three lakes studied in which *Chaoborus* increased during the 1960's (Fig. 4). The untreated basin is very close (100 m) to the treated basin, making *Chaoborus* recolonization of the treated basin potentially quite high. Thus, residual toxaphene might at first appear to have been a cause for poor recruitment of chaoborids. However, in addition to receiving a lower toxaphene dose than Chatwin Lake, and having low populations of *Chaoborus* prior to treatment, it is possible that fathead minnow and brook stickleback (abundant in the treated basin of Peanut Lake at present) were not completely eliminated, or were reintroduced soon after poisoning. If so, then continuous fish predation could also be considered a cause of low *Chaoborus* populations from pretreatment years to present. We are not sure why a similar control was not exerted in the untreated basin. Unfortunately, except for the probability that few or no fish remain because of low oxygen concentrations (AFW files), no specific records of fish populations in the untreated basin exist. Specific knowledge of the fish populations in both basins of Peanut Lake would further elucidate the relative contributions of predation by fish, toxaphene toxicity, and probably other variables on *Chaoborus* abundance.

In Chatwin Lake, total chironomid abundance was apparently unaffected by toxaphene application. Chironomids have been observed to become depleted but to increase more quickly than other invertebrates after toxaphene application (Stringer and McMynn 1958; Webb 1980), a response that was partly attributed to removal of benthivorous fish (Webb 1980). In another case, chironomids were absent for 9 mo from samples in a lake treated with five times the toxaphene dose that Chatwin Lake received (Cushing and Olive 1957). Thereafter, they repopulated the lake. Our subsamples for dipterans may combine remains from more than one year because several laminae are usually required to make up the necessary sample size (3 mL). This would obscure short-term responses of dipterans that may have been affected for only 1 or 2 yr, resulting in the conservative conclusion that total chironomid abundance was unchanged by toxaphene treatment.

It is possible that further taxonomic characterization of the chironomid fauna might reveal sensitivity of specific taxa. In his monitoring study in Ontario, Webb (1980) found that members of the subfamily Orthocladiinae and *Chironomus cucini* only reappeared 4 yr after toxaphene treatment, that *Phaenopsectra* (*Sergentia*) *coracina* and *Procladius freemani* recovered after 1 yr, and that one species (*Cryptotendipes casuarius*) that had not occurred in the lake before appeared in the lake 2 yr after treatment. He noted that overall chironomid abundance was reduced for only one season and that significant increases occurred thereafter. Because chironomid head parts usually preserve well enough in sediments for generic identification (Walker 1987), further taxonomic work might reveal a genera-dependent response of chironomids in lakes that were treated with toxaphene.

Long-term (Post-toxaphene) Recovery

A change from small to large invertebrates is expected when the abundance of planktivorous fish is reduced (Brooks and Dodson 1965; Shapiro and Wright 1984; Carpenter et al. 1985; Leavitt et al. 1989). Because large invertebrates replaced smaller ones in Chatwin Lake and large invertebrates increased greatly in both basins of Peanut Lake (Fig. 4), we infer reduced planktivory as the principal explanation. Reduced numbers of planktivores are consistent with government records that indicate poor trout catches in test nets despite intensive stocking in the two treated lakes (AFW files).

Low levels of zooplanktivory by stocked or reintroduced fish populations have also been observed in lakes that were treated with rotenone. For example, the shift to dominance by large-bodied zooplankton has occurred in other rainbow trout stocked lakes that were first treated with rotenone (Anderson 1970; Leavitt et al. 1989). All except one crustacean species reached prerotenone abundances in about 3 yr in two mountain lakes (Anderson 1970). Large herbivores apparently recovered immediately and remained dominant after rotenone treatment and during trout stocking in Paul Lake, Michigan (Leavitt et al. 1989).

One clue as to the possible presence of planktivores (briefly mentioned in the previous section) in the untreated basin of Peanut Lake is the absence of *C. americanus* in sediments since basin division (Fig. 4c). *Chaoborus americanus* was detected in the 1940's in the untreated basin of

Peanut Lake and would be expected to reappear in the complete absence of predatory fish (Pope et al. 1973; Johnson et al. 1990). A species that does coexist with planktivores (*C. flavicans*, Johnson 1990) became extremely abundant in the untreated basin in the past 20 yr, suggesting planktivores may have persisted despite depleted oxygen during the winter. The presence of reestablished fathead minnow and brook stickleback populations in the treated basin (AFW files) and this evidence of residual populations in the untreated basin would likely explain many of the similarities found in the invertebrate remains in both basins.

Increases in both the abundance of invertebrates (Fig. 4b and 4c) and sediment organic matter (Fig. 3) suggest that Peanut Lake became more productive from the 1960's onward. In contrast, in Chatwin Lake, chironomid abundance (Fig. 4a) and percent organic matter did not vary much over the 50-yr history, suggesting that productivity has not changed substantially in this lake during the period of study. The lack of change in organic matter in Chatwin Lake despite the much higher dose and sediment concentrations of toxaphene precludes implications of long-term toxicity to bacterial decomposers. Increased percent organic matter of sediments may be inferred as higher productivity because of either an increase in nutrient inputs or longer water residence times (Dillon 1975; Schindler et al. 1978; Whiteside 1983). Water residence times in similar small seepage lakes in central Alberta are estimated to exceed 100 yr (Mitchell and Prepas 1990), making it unlikely that altering this factor by even several years would contribute to changes in production. Increased nutrient loading may have been caused by the farming operation that encompasses about one third of the shoreline of Peanut Lake and by additional public use of the treated basin in the past 20 yr.

Predation by invertebrates may have contributed to the long-term depletion of the small-bodied herbivores in Chatwin Lake. *Daphnia rosea* were greatly reduced and *Bosmina* were eliminated coincident with rises in predatory *C. americanus* in Chatwin Lake during the 1970's (Fig. 4a). This chaoborid is an effective predator on small zooplankton (Elser et al. 1987). In some cases, large populations of *Chaoborus* spp. have been known to decimate (Von Ende and Dempsey 1981), or at least strongly regulate (Yan et al. 1991), *Bosmina* populations. Clearly, *Bosmina* never recovered from toxaphene-induced depletion in Chatwin Lake and probably was ultimately eliminated by intense invertebrate predation (Fig. 4a, mid-1970's). Other explanations including competition or other factors cannot be applied because they are difficult enough to establish based on contemporary records and are too speculative to infer from the sedimentary record.

In summary, the short-term responses to toxaphene treatment of the invertebrate communities in two treated lakes differed, probably mainly due to different toxaphene dosages. Toxicity was apparent for some organisms in the lake with a higher toxaphene dose, but was not detected in the lake with the lower dose. Long-term effects on invertebrates after treatment and stocking of the lakes were similar in many ways and were probably the result of less intense planktivory after treatment, despite repeated stocking with rainbow trout.

The information gained from sediment cores from these lakes would otherwise have been lost because of the lack of contemporary monitoring data. The paleolimnological methods used in this study could easily be applied to other suitable lakes to reconstruct the effects of perturbations of many kinds.

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Induced Cytochrome P4501A in Winter Flounder, *Pleuronectes americanus*, from Offshore and Coastal Sites

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Cytochrome P4501A (CYP1A), Aroclor 1254 (A1254), and 3,3′,4,4′-tetrachlorobiphenyl (TCB) were measured in liver of winter flounder, *Pleuronectes americanus*, from Boston Harbor, Mass., Hempsted Harbor, N.Y., Niantic, Conn., and an offshore site, Georges Bank. We also measured CYP1A content and activity in flounder from Passamaquoddy Bay, N.B. Concentrations of A1254 and TCB were the least in fish from Georges Bank (0.46 and 0.002 $\mu g \cdot g$ dry weight^{-1}, respectively); concentrations in fish from Boston, Niantic, and Hempsted ranged from 7.6 to 11.3 $\mu g \cdot g^{-1}$ and from 0.013 to 0.024 $\mu g \cdot g^{-1}$. Immunodetected microsomal CYP1A contents (expressed as scup P450E equivalents) were 0.17 and 0.19 $nmol \cdot mg^{-1}$ in fish from Georges Bank and Passamaquoddy and 0.25–0.41 $nmol \cdot mg^{-1}$ in fish from Boston, Niantic, and Hempsted. Ethoxyresorufin-*O*-deethylase specific activities likewise were greater in fish from Boston, Niantic, and Hempsted (1.7–2.4 $nmol \cdot min^{-1} \cdot mg^{-1}$) than in fish from Georges Bank or Passamaquoddy (0.83 and 0.61 $nmol \cdot min^{-1} \cdot mg^{-1}$). CYP1A content and activity were correlated with hepatic concentrations of A1254 and TCB. These data, together with data reported in previous studies, indicate that strong induction of CYP1A protein occurs in winter flounder populations along most of the industrialized east coast and that induction of CYP1A is common, but less strong, at sites distant from the urban centers of the Northeast.

Le cytochrome P4501A (CYP1A), l'Aroclor 1254 (A1254) et le 3,3′,4,4′-tétrachlorobiphényle (TCB) ont été dosés dans le foie de plies rouges provenant du port de Boston, Mass., de Hempsted Harbor, N.Y., de Niantic, Conn., ainsi que d'une station offshore, le banc Georges. Nous avons aussi mesuré la teneur et l'activité du CYP1A dans la plie rouge de la baie Passamaquoddy, N.-B. La concentration en A1254 et en TCB était le moins élevée dans le poisson du banc Georges (0,46 et 0,002 $\mu g \cdot g$ poids sec^{-1}, respectivement). La concentration dans le poisson provenant des secteurs de Boston, de Niantic et d'Hempsted variait entre 7,6 et 11,3 $\mu g \cdot g^{-1}$ et entre 0,013 et 0,024 $\mu g \cdot g^{-1}$. La teneur en CYP1A microsomial mesurée par immunodétection (et exprimée en équivalents de P450E dans le spare doré) était 0,17 et 0,19 $nmol \cdot mg^{-1}$ dans le poisson du banc et de la baie Passamaquoddy et entre 0,25 et 0,41 $nmol \cdot mg^{-1}$ dans le poisson qui provenait de Boston, de Niantic et d'Hempsted. De la même façon, l'activité spécifique de l'éthoxyrésorufine-*O*-déséthylase était spérieure dans la chair du poisson provenant de Boston, Niantic et Hempsted (1,7–2,4 $nmol \cdot min^{-1} \cdot mg^{-1}$) que dans la chair du poisson provenant du banc Georges ou de la baie Passamaquoddy (0,83 et 0,61 $nmol \cdot min^{-1} \cdot mg^{-1}$). La teneur en CYP1A et son activité étaient en corrélation avec la concentration dans le foie de l'A1254 et du TCB. Ces résultats, et d'autres données rapportées dans des études antérieures montrent qu'il existe une forte induction de la protéine CYP1A dans les populations de plies rouges sur la majeure partie de la côte est industrialisée, et que l'induction de la CYP1A est communément observée, mais moins intense, aux stations éloignées des centres urbains du nord-est.

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In vertebrates from fish to mammals, liver microsomal cytochrome P450 forms belonging to the P4501A (CYP1A) subfamily (Nebert et al. 1991)[3] can be induced by common environmental contaminants such as polynuclear aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), dibenzofurans, and dioxins (Safe 1984; Gonzalez 1989; Stegeman 1989). This induction is apparently mediated by the Ah receptor (Poland and Knutson 1982). Ah receptor proteins have been identified in liver of a variety of fishes (Heilman et al. 1988; Lorenzen and Okey 1990; Hahn and Stegeman 1992). Hepatic CYP1A induction is being used increasingly as a marker of exposure to environmental contaminants that can bind the receptor. In judging the significance of CYP1A induction, it is important to determine the causes and extent of induction in the environment.

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[3]Proteins that cross-react with monoclonal antibody 1-12-3 are tentatively assumed to be CYP1A proteins (Stegeman 1992).

FIG. 1. Winter flounder collection sites. a = Hempsted Harbor (HH), b = Niantic (N), c = Boston Harbor (BH), d = Georges Bank (GB), e = Passamaquoddy Bay (this study) (PQ), f,g = Gaspee Point (GP), Fox Island (FI), h = New Bedford Harbor (NBH) (Elskus et al. 1992), i = Narrow River (NR), j = Georges Bank (Monosson and Stegeman 1991), k = Buzzards Bay (BB), l = Nantucket Sound (NA), m = Plymouth Bay (PB), n = Boston Harbor (BH) (Stegeman et al. 1987), p = Mt. Desert Island (ME) (Foureman et al. 1983), o = St. Margarets Bay (SM) (Edwards et al. 1988).

Environmental induction of monooxygenease activity in fish liver has been studied for more than 15 yr (reviewed in Payne et al. 1987; Goksoyr and Forlin 1992; Stegeman et al. 1992). Elevated levels of hepatic CYP1A catalytic activity, increased CYP1A protein, and increased CYP1A mRNA have been associated with PAH and/or PCB exposure in several studies (Stegeman et al. 1986; Varanasi et al. 1986; Elskus and Stegeman 1989; Van Veld et al. 1990). Laboratory studies with specific PCB congeners have shown that the nonortho-substituted planar structures (e.g., 3,3′,4,4′-tetrachlorobiphenyl (3,3′,4,4′-TCB; IUPAC No. 77)) are highly active in CYP1A induction in fish (Melancon and Lech 1983; Gooch et al. 1989; Monosson and Stegeman 1991). Accordingly, planar compounds such as 3,3′,4,4′-TCB or others that may co-occur with the PCBs are suspected of being active environmental inducing agents in many fish.

In this study, we measured the content and activity of CYP1A in liver of winter flounder, *Pleuronectes americanus*, populations over a large geographic region ranging from New York, U.S.A., to New Brunswick, Canada, and investigated the relationship between content and activity of CYP1A and the concentration of PCBs in livers of flounder from sites along the New York and New England coast and Georges Bank. This comparison was the outgrowth of our search for flounder that would show at most slight indication of exposure to Ah receptor agonists or other contaminants. Locations of such "reference" populations would be useful in helping to establish the etiology of hepatic neoplastic and other diseases of flounder living in the vicinity of contaminated sediments. Flounders in some locales bear neoplastic and other histological diseases (Murchelano and Wolke 1985) that are thought to derive from environmental contamination.

Materials and Methods

Collection and Preparation of Samples

Winter flounder populations are relatively localized, as their migrations are mainly limited to onshore–offshore movements, especially those north of Cape Cod (Howe and Coates 1975). Flounder south of the Cape exhibit a more southeastward dispersion. Movements were reported to be confined to an average of 10 mi (1 mi = 1.61 km), with a greatest straight-line distance of 54 mi (Saila 1961). In either case, there is little or no mixing of coastal groups with fish from Georges Bank. For these reasons, winter flounder is an appropriate species for broad geographical monitoring programs (e.g., interestuary studies).

Flounder (27–37 cm) were collected by otter trawl or hook and line from four coastal sites between February and June. Flounder were collected from three highly to moderately populated sites (Boston Harbor, Mass., Hempsted Harbor, N.Y., and Niantic, Conn.) with relatively high sediment concentrations of PCBs and PAHs (NOAA 1988) and from Passamaquoddy Bay, N.B., which is less populated than the former sites (Fig. 1). Companion animals were collected at these sites (except for Passamaquoddy Bay) for PCB analysis. We also collected flounder from one offshore site, Georges Bank, distant from coastal pollution sources with relatively little PCB or PAH sediment contamination (Backus 1978). We recently showed (Monosson and Stegeman 1991) that Georges Bank flounder had CYP1A content and response to inducers indicative of fish that had low basal expression of CYP1A.

Flounder from Boston, Hempsted, and Niantic were killed within a few hours of capture. Flounder from Passamaquoddy Bay were held for only 1 wk under flow-through conditions at the St. Andrews Biological Station, St. Andrews, N.B., and killed at the station. Flounder from Georges Bank (41.3N, 68.2W) were collected by trawling (average trawl speed was 2.5 kn with 30-min trawls). Because of limited holding capacity onboard ship, the average length of flounder brought back to the laboratory for CYP1A analysis was 30 cm. Somewhat larger Georges Bank flounder (38 cm), collected 2 wk later from the same site, were used for chemical analysis. These fish were held under the same conditions and killed within 2–3 d upon return to the laboratory. Larger, older flounder from some sites tend to have higher body burdens of PCBs (R. Pruell, USEPA, Narragansett, R.I., personal communication). However, we could not detect size-related differences in either CYP1A activity or heptatic PCB concentrations for Georges Bank flounder ranging from 28 to 38 cm (unpublished observations).

Fish were killed by cervical transection. Gallbladders were removed to avoid contaminating the liver with bile.

TABLE 1. Body measurements (mean ± SD) of winter flounder from coastal sites and Georges Bank. ND, no data.

Site	Date collected	*N*	Weight (g)	Length (cm)	GSI[a]	
Passamaquoddy Bay	February	7	198 ± 49	ND	Male (5)	5.4 ± 1.0
					Female (2)	0.9
Georges Bank	June	7	328 ± 55	30 ± 2	Male (4)	0.1 ± 0.1
					Female (3)	0.5 ± 0.5
Boston Harbor	May	10	682 ± 271	37 ± 4	Male (6)	0.6 ± 0.2
					Female (4)	2.0 ± 0.4
Niantic River	April	5	206 ± 32	27 ± 2	Male (3)	1.0 ± 0.2
					Female (2)	1.3
Hempsted Harbor	May	5	124 ± 17	23 ± 1	Male (5)	Regressed

[a]Number of animals for GSI in parentheses.

Livers were weighed and frozen in liquid nitrogen. Livers removed for chemical analysis were stored in aluminum foil or glass jars at −20°C until analysis. Microsomes were prepared according to established methods (Stegeman et al. 1979) and stored in liquid nitrogen. Gonadal and hepatic somatic indices (GSI and HSI) were obtained by calculating the percent body weight due to gonad or liver weight. Protein was determined using the bicinchoninic acid (BCA) protein assay (Smith et al. 1985). All chemicals used in this study were of the highest grade available from commercial suppliers.

Enzyme Analysis

Hepatic cytochrome P450 content was determined using a dithionite difference spectrum based on the methods of Omura and Sato (1964) and modified as described in Stegeman et al. (1979). Ethoxyresorufin-*O*-deethylase (EROD) activity was determined using a visible spectrophotometric assay according to the method of Klotz et al. (1984). 7-Ethoxyresorufin was synthesized in this laboratory as described in Klotz et al. (1984). Western blots were used to quantify microsomal CYP1A content with slight modifications of the methods described in Kloepper-Sams et al. (1987) and Kloepper-Sams and Stegeman (1992) using the anti-scup, *Stenotomus chrysops*, CYP1A monoclonal antibody (Mab) 1-12-3; Mab 1-12-3 is cross-reactive with CYP1A in the winter flounder (Stegeman et al. 1987). Secondary antibody and color development were accomplished as before (Kloepper-Sams et al. 1987) and blots were scanned using a soft laser densitometer (Helena Labs, Beaumont, Tex.) and quantified using purified scup CYP1A standards, assuming a similar degree of reactivity with Mab 1-12-3 as seen with scup P450E.

Chemical Residue Analysis

Flounder liver samples were collected from different individuals than those used for CYP1A analysis. PCBs were extracted using a liquid/liquid extraction technique (Pruell et al. 1988). All solvents for GC-ECD were purchased from Baxter Scientific, McGraw Park, Ill. Prior to extraction, hepatic tissue samples (0.3–1.0 g) were freeze-dried overnight in order to obtain accurate wet-to-dry ratios. Samples were homogenized in acetone (25 mL × 3) (following addition of octa-chloronaphthalene as an internal standard). After centrifugation, the acetone supernatant was combined with freon-rinsed water (150 mL). PCBs were extracted from the acetone–water mixture with freon (25 mL × 3), volume reduced, and solvent exchanged with hexane. Samples were digested overnight with concentrated sulfuric acid and solvent exchanged with heptane (final sample volume 1.0 mL). Planar congeners were separated from the extracted PCB mixture using activated carbon/silica columns (2.5-mm columns with 100-g silica gel layered with 5 g of activated carbon); nonplanar compounds were eluted with methylene chloride (10 mL), the columns were reversed, and planar compounds were eluted with toluene (5 mL). Sample volumes were reduced to a final volume of 0.3 mL under a stream of N_2 and analyzed for total Aroclor 1254 (A1254), 3,3′,4,4′-TCB, 2,3′,4,4′,5-pentachlorobiphenyl (2,3′,4,4′,5-PCB; IUPAC No. 118), and 2,2′,3,4,4′,5′-hexachlorobiphenyl (2,2′,3,4,4′,5-HCB; IUPAC No. 138) by GC–ECD chromatography.

Statistical Analysis

Because of unequal sample sizes, we examined the data using least squares means (SAS, SAS Institute, Cary, N.C.). Comparisons among the means were considered significant at $p < 0.05$.

Results

Sites from which winter flounder were collected for the present and for previous studies in the same region (see below) are shown in Fig. 1. The Niantic, Boston, and Hempsted sites are all adjacent to highly populated coastal regions known to have relatively high sediment concentrations of PAHs and PCBs, unlike Passamaquoddy Bay or Georges Bank. Dates of collection and sizes of animals for P450 analysis are given in Table 1. As there were no gender-related differences in CYP1A content or activity, data for males and females were combined.

There were large differences in the mean content of total microsomal P450 and CYP1A content and activity between flounder from different sites. Total P450 content was less in flounder from the Passamaquoddy Bay, Georges Bank, and Boston sites than in fish from other sites (Table 2). The highest total P450 contents measured, in flounder from Niantic, were about twofold greater than P450 contents in Georges Bay or Passamaquoddy Bay fish (0.74 nmol·mg^{-1} as compared with 0.35 nmol·mg^{-1}). Rates of EROD specific activity in flounder collected from each of the five sites also differed (Table 2). Flounder from Passamaquoddy Bay and Georges Bank had the lowest EROD activity, while

TABLE 2. Cytochrome P4501A content and activity (mean ± SD) in winter flounder (males and females combined, $N = 5–10$) from coastal sites and Georges Bank. Values followed by a different letter are significantly different ($p = 0.05$).

Site	P450 (nmol·mg^{-1})	CYP1A (nmol·mg^{-1})	EROD (nmol·mg^{-1}·min^{-1})	Turnover) (EROD·nmol P4501A^{-1})
Passamaquoddy Bay	0.35 ± 0.21 A	0.17 ± 0.11 A	0.61 ± 0.44 A	3.9 ± 2.3 A
Georges Bank	0.35 ± 0.18 A	0.19 ± 0.18 A	0.83 ± 0.27 A	5.0 ± 1.3 A
Boston harbor	0.48 ± 0.24 AB	0.25 ± 0.12 A	1.70 ± 1.28 B	6.3 ± 2.4 A
Niantic River	0.74 ± 0.18 C	0.29 ± 0.05 AB	1.96 ± 1.20 B	6.5 ± 3.0 A
Hempsted Harbor	0.62 ± 0.19 BC	0.41 ± 0.12 B	2.36 ± 0.58 B	6.6 ± 4.1 A

TABLE 3. Concentrations (μg·g dry weight^{-1}, mean ± SD) of A1254 and selected PCB congeners present in liver tissue of winter flounder collected from coastal sites and Georges Bank.

Site	N	Aroclor 1254	3,3′,4,4′-TCB	2,3′,4,4′,5-PCB[b]	2,2′,3,4,4′,5′-HCB[b]	Reference[c]
Georges Bank[a]	4	0.46 ± 0.22	0.002 ± 0.001	0.046 ± 0.037	0.061 ± 0.054	1
Boston Harbor	4	7.64 ± 2.85	0.013 ± 0.006	0.280 ± 0.071	0.398 ± 0.107	1
Niantic River	5	10.72 ± 4.67	0.008 ± 0.004	0.251 ± 0.109	0.434 ± 0.189	1
Hempsted Harbor	5	11.30 ± 6.04	0.024 ± 0.013	0.472 ± 0.198	0.593 ± 0.244	1
Fox Island	6	4.05 ± 2.63	0.004 ± 0.003	0.135 ± 0.092	0.174 ± 0.121	2
Gaspee Point	6	13.25 ± 10.02	0.005 ± 0.005	0.370 ± 0.244	0.481 ± 0.324	2
New Bedford Harbor	6	81.71 ± 104.91	0.267 ± 0.440	7.798 ± 8.276	4.930 ± 4.620	2

[a]Two Georges Bank samples were below detection limits for 3,3′,4,4′-TCB. Detection limits were calculated for each sample as follows: 1.5 ng 3,3′,4,4′-TCB·g dry weight liver^{-1}; this accounts for the congener detection limit (1.5 pg·μL^{-1}). One-half the detection limit was used to calculate the means for these samples.

[b]$N = 3$ for Georges Bank and $N = 4$ for Hempsted Harbor.

[c]1 = This study; 2 = Elskus et al. (1993).

the highest average EROD activity was found in flounder from Hempsted Harbor. There was a large amount of variation in total P450 content and CYP1A content and activity, most evident in fish from Boston Harbor. However, between 40 and 65% of the total P450 appeared to be CYP1A for individual fish from each site, assuming equal activity of scup and flounder CYP1A with Mab 1-12-3 (data not shown).

Hepatic CYP1A content in flounder from Passamaquoddy Bay and Georges Bank (0.17 and 0.19 nmol·mg^{-1}) was approximately one half that in flounder collected from Hempsted Harbor (Table 2). The Hempsted Harbor fish had the highest CYP1A content (0.41 nmol·mg^{-1}). Studies of CYP1A in other fish (e.g., Klotz et al. 1983) and correlations previously shown in winter flounder (Stegeman et al. 1987) indicate that CYP1A is the EROD catalyst in flounder. We therefore calculated turnover number (EROD activity per nanomole of P4501A enzyme). There were no site-related differences in the estimated turnover number.

PCB Residues

The hepatic concentrations of A1254 and three PCB congeners from flounder collected at the same sites as those used for P450 analysis are shown in Table 3 together with data from Elskus et al. (1992) for comparison. Flounder from Georges Bank had concentrations of A1254 (0.46 μg·g dry weight^{-1}) and 3,3′,4,4′-TCB, (0.002 μg·g dry weight^{-1}) less than those in fish from the other three sites for which PCBs were measured. Hempsted Harbor flounder had the greatest concentrations of both A1254 (11.3 μg·g^{-1}) and 3,3′,4,4′-TCB (0.024 μg·g^{-1}) of all four of our sites. Concentrations of the monoortho-substituted 2,3′,4,4′,5-PCB and the diortho-substituted 2,2′,3,4,4′,5′-HCB tended to be 30- to 100-fold higher than concentrations of 3,3′,4,4′-TCB.

The mean A1254 concentrations for the populations studied appeared to be positively correlated with mean CYP1A content and EROD activity ($r = 0.83$, $p = 0.17$ and $r = 0.96$, $p = 0.04$) in flounder collected from all sites (Fig. 2A and 2B). Likewise 3,3′,4,4′-TCB concentrations also appeared to be positively correlated with CYP1A content and activity and 3,3′,4,4′-TCB ($r = 0.92$, $p = 0.08$ and $r = 0.85$, $p = 0.15$) (Fig. 2C and 2D). The correlations, although evident, were statistically nonsignificant at $p = 0.05$ (except between A1254 and EROD activity), likely due to the small number of sites sampled.

Flounder from Passamaquoddy Bay were collected just prior to spawning season in February. Although we have evidence of low hepatic A1254 concentrations in those fish (A1254 = 0.54 μg·g^{-1} in fish held for 4 wk (E. Monosson and J.J. Stegeman, unpublished)), the relatively low CYP1A content and activity should be regarded with caution, as there is evidence that seasonal and/or temperature changes can affect EROD activity in flounder from relatively clean sites (Edwards et al. 1988). Gonadally mature (recrudescent) and prespawning female flounder have decreased EROD activity and CYP1A content compared with recrudescent or nonrecrudescent males (which show little difference) or with females that do not have mature gonads (Stegeman and Woodin 1984; Gray et al. 1991). The GSI for females from Passamaquoddy Bay was 0.9% (Table 1), well below GSIs observed in typical gonadally mature female flounder, which can be as high as 15% or more. Low water temperatures (4°C) can also suppress CYP1A induction in some fish species (Jiminez and Burtis 1988), and EROD activities tended to be lower in flounder collected in the winter as compared with those collected in the summer from St. Margaret's Bay (Edwards et al. 1988). However, strong

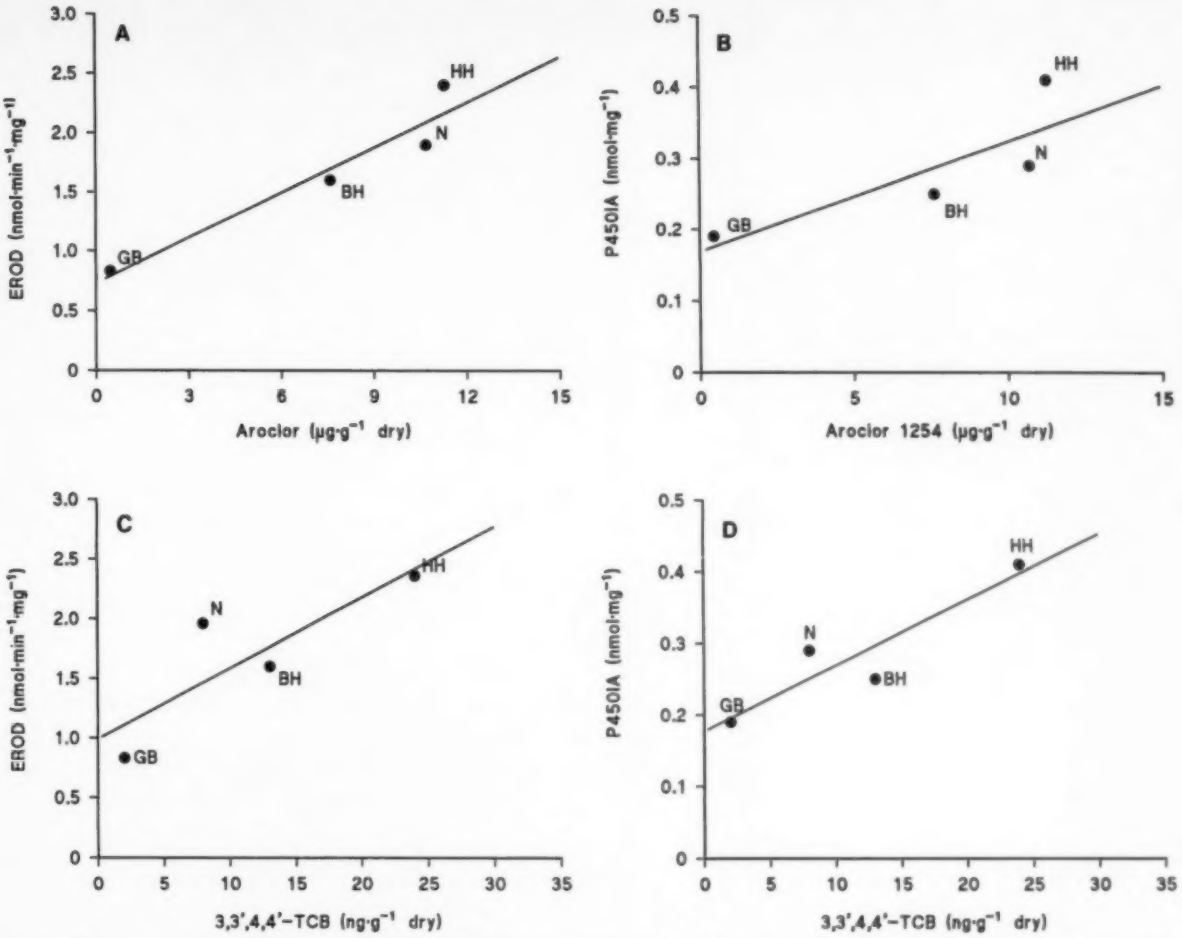

FIG. 2. Relationships between population mean CYP1A content and activity and mean A1254 in winter flounder from three coastal sites and from Georges Bank. (A) EROD activity versus A1254; (B) CYP1A content versus A1254; (C) EROD activity versus 3,3′,4,4′-TCB; (D) CYP1A content versus 3,3′,4,4′-TCB. N = 5–10 fish per site (males and females combined) for CYP1A measurements. Flounder used for chemical analysis were collected concurrently with flounder collected for the CYP1A studies; N = 4–5 for each site. Site abbreviations are given in Fig. 1.

induction of CYP1A activity has been observed in flounder acclimated to cold (4–9°C) temperatures (E. Monosson and J.J. Stegeman, unpublished). Induction at low temperatures has also been demonstrated in *Fundulus* (Kloepper-Sams and Stegeman 1992) and rainbow trout, *Oncorhynchus mykiss* (Forlin et al. 1984). This relationship between CYP1A induction and seasonal effects (including both degree of gonadal development and ambient water temperature) in flounder requires further investigation.

Discussion

Environmental induction of hepatic CYP1A is increasingly recognized as a common phenomenon in fish from fresh and marine waters. Interpreting the significance of that CYP1A induction will depend in part of the identity and concentration of inducers to which animals were exposed. In this study, we used livers from individual fish for cytochrome P450 analysis, and collected additional fish for chemical analysis, to investigate the relationship between hepatic concentrations of PCBs and CYP1A induction among different populations. The results indicated a positive correlation between population mean content of CYP1A and mean concentration of PCB residues in liver of winter flounder from three coastal sites and Georges Bank.

The rank order for A1254 concentrations in flounder liver was similar to the rank order reported for sediments at the sites where we sampled fish (Backus 1987; NOAA 1988). Although we examined induction and PCB concentrations in companion fish and not in the same animals, the hepatic CYP1A content and activity in those fish reflect the differing degrees of contamination. Other studies of fish along known pollution gradients have indicated relationships between the degree of CYP1A induction in liver and concentrations of residues of PCBs or PAHs in sediments (Elskus and Stegeman 1989; Van Veld et al. 1990), bivalve mollusks taken at the same site (Stegeman et al. 1988; Narbonne et al. 1991), and/or livers (Elskus 1992; Elskus et al. 1993)

TABLE 4. Cytochrome P450 measurements (mean ± SD) in male winter flounder reported in other studies.

Site	Month	P450 content ($nmol \cdot mg^{-1}$)	EROD ($nmol \cdot mg^{-1} \cdot min^{-1}$)	CYP1A content ($nmol \cdot mg^{-1}$)	Turnover number ($EROD \cdot CYP1A^{-1}$)	Reference[f]
Passamaquoddy Bay	Feb.	0.35 ± 0.21	0.61 ± 0.44	0.17 ± 0.11	3.9	1
St. Margarets Bay[a]	Jan.–Dec.	0.28 ± 0.18	0.58 ± 0.47	—	—	2
Georges Bank	June	0.35 ± 0.18	0.83 ± 0.27	0.19 ± 0.08	5.0	1
Georges Bank[b]	June	0.51 ± 0.14	0.16 ± 0.14	0.01 ± 0.01	14.1	3
Mt. Desert, Maine[c]	June–Aug.	0.26 ± 0.06	0.28 ± 0.07	—	—	4
Boston Harbor, Mass.	May	0.48 ± 0.24	1.61 ± 1.00	0.25 ± 0.12	9.2	1
Boston Harbor, Mass.[d]	June–Oct.	0.62 ± 0.06	1.19 ± 0.11	0.29 ± 0.03	4.1	5
Plymouth Bay, Mass.[d]	June–Oct.	0.67 ± 0.07	1.27 ± 0.14	0.41 ± 0.09	3.1	5
Nantucket, Mass.[d]	June–Oct.	1.04 ± 0.13	0.87 ± 0.12	0.46 ± 0.10	1.9	5
Buzzards Bay, Mass.[d,e]	June–Oct.	0.90 ± 0.90	0.78 ± 0.39	0.17 ± 0.23	3.8	5
New Bedford, Mass.	Feb.–Mar.	0.51 ± 0.32	1.91 ± 0.60	0.43 ± 0.08	3.4	6, 7
Gaspee Point, R.I.	Feb.–Mar.	0.79 ± 0.25	2.02 ± 0.82	0.39 ± 0.05	6.6	6, 7
Fox Island, R.I.	Feb.–Mar.	0.76 ± 0.39	1.63 ± 1.44	0.41 ± 0.19	3.4	6, 7
Narrow River, R.I.	Nov.	1.79 ± 0.44	2.02 ± 1.44	0.82 ± 0.30	1.7	3
Niantic River, Conn.	Apr.	0.74 ± 0.18	1.96 ± 1.20	0.29 ± 0.05	6.5	1
Hempsted Harbor, N.Y.	May	0.62 ± 0.19	2.36 ± 0.58	0.41 ± 0.12	6.6	1

[a]Average over 2 yr of monthly sampling. Edwards et al. (1988) reported lower CYP1A activities in the winter as compared with the summer.

[b]These fish were held at 15°C for 8–10 d before killing, which could have resulted in metabolism of PAHs and a decline in CYP1A protein content and activity (Monosson and Stegeman 1991).

[c]Sex unknown. The means were calculated from individual data points in Foureman et al. (1983). The EROD are for two different groups of Mt. Desert flounder and were measured using fluorometric techniques.

[d]Males and females (authors reported no sex difference in P450 content or activity).

[e]Separating this group ($N = 8$) into samples with inhibition by α-naphthoflavone above or below 50% results in two groups of $N = 4$, with CYP1A content and turnover number equal to 0.3 $nmol \cdot mg^{-1}$ and 3.9 $nmol \cdot min^{-1} \cdot nmol^{-1} \cdot CYP1A^{-1}$ (high group) and 0.035 $nmol \cdot mg^{-1}$ and 3.5 $nmol \cdot min^{-1} \cdot nmol^{-1} \cdot CYP1A^{-1}$ (low group) (Stegeman et al. 1987).

[f]1 = This study; 2 = Edwards et al. (1988); 3 = Monosson and Stegeman (1991); 4 = Foureman et al. (1983); 5 = Stegeman et al. (1987); 6 = Elskus et al. (1989); 7 = A.A. Elskus (Ph.D. dissertation, 1992). There are interlaboratory differences in the techniques used to measure EROD activity; an interlaboratory comparison of EROD activity in fish has been conducted by Munkittrick et al. (1993).

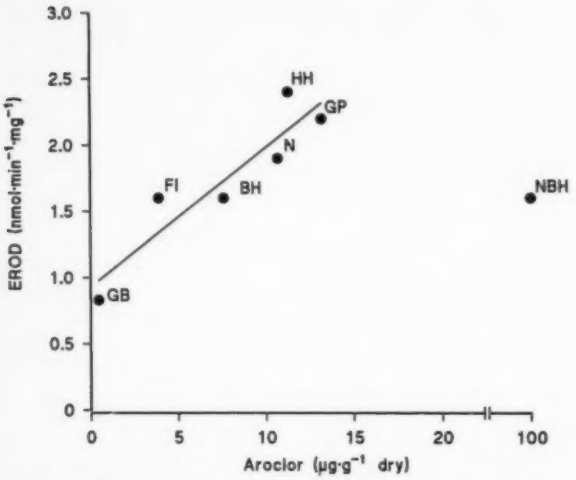

FIG. 3. Relationship between mean EROD activity versus mean A1254. Data presented are from this study (GB, BH, N, HH) and from Elskus et al. (1989) and A.A. Elskus (Ph.D. dissertation, 1992) (FI, GP, NBH). Line of best fit is to all sites excluding NBH. Site abbreviations are given in Fig. 1.

or bile (PAH metabolites) (Varanasi et al. 1986) of fish analyzed for CYP1A. Clearly, environmental chemical inducers could include PAHs, as well as other planar halogenated aromatic hydrocarbons. PAHs are rapidly metabolized in fish, so that a strong positive relationship between PAH residues and induction cannot be expected. However, close relationships between PAH metabolites in bile and induction of CYP1A in liver have been observed (Varanasi et al. 1986). Unlike PAHs, most PCB congeners are not rapidly metabolized and are therefore persistent in fish tissues (Hutzinger et al. 1972; Nimmi and Oliver 1983). A closer relationship to CYP1A induction in fish may therefore be expected.

In fish studied to date, the nonortho PCB congeners are strong inducers, but the monoortho- or diortho-substituted congeners are weakly active or inactive in induction (Melancon and Lech 1983; Gooch et al. 1989; Skaare et al. 1991) or toxicity in fish (Walker and Peterson 1991). Thus, even in environments where PCBs are abundant, the degree of CYP1A induction might be expected to be more closely related to the content of planar, nonortho-substituted PCB congeners (e.g., 3,3′,4,4′-TCB and 3,3′,4,4′,5-PCB) than to total PCB concentration even though the monoortho- and diortho-substituted congeners are present at much greater concentrations.

Comparing the relationships between CYP1A content or activity and PCB concentrations with similar results from other studies of flounder in New England waters provides a larger picture of the correlation with possible inducers. The data here and those of Elskus et al. (1989, 1993) together show a strong positive linear correlation ($r = 0.94$, $p = 0.004$) between population means of CYP1A and PCB content in flounder where hepatic mean concentrations of A1254

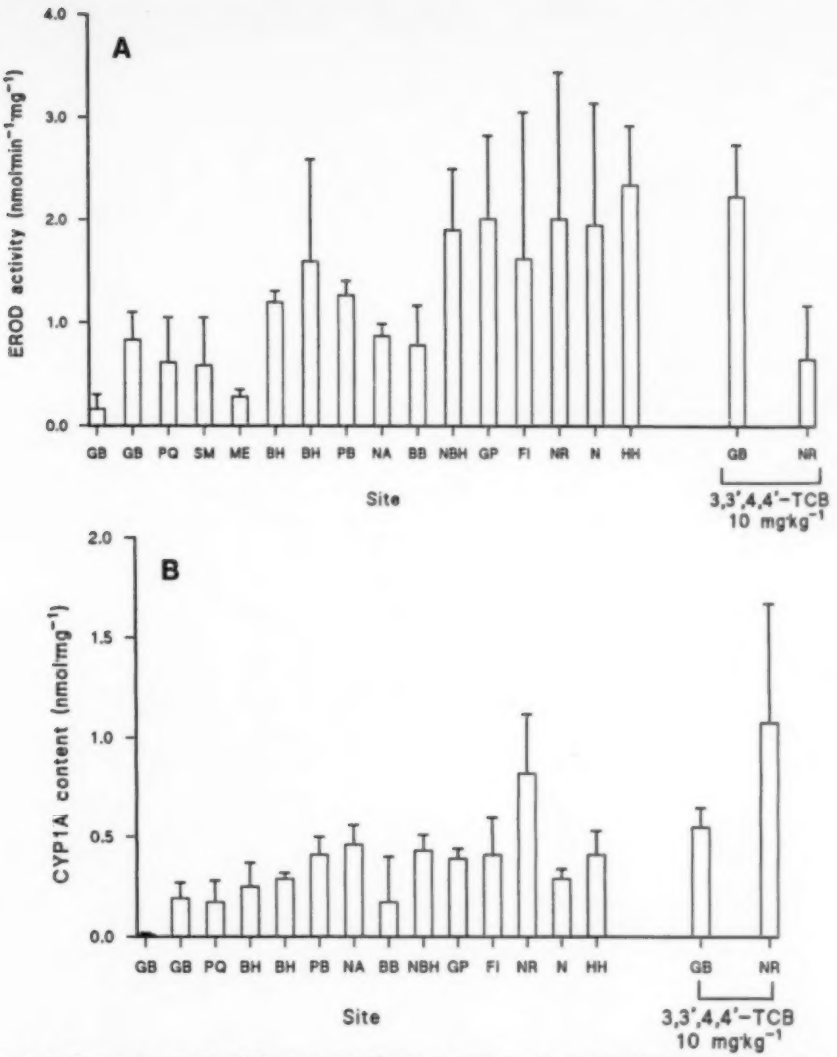

FIG. 4. Composite of (A) EROD and (B) CYP1A content in winter flounder collected at various east coast sites (mean ± SD). Data are from this study (PQ, GB, N, HH, BH), Monosson and Stegeman (1991) (NR, GB), A.A. Elskus (Ph.D. dissertation, 1992) (GP, FI, NBH), Stegeman et al. (1987) (BH, BB, PB, NA), Foureman et al. (1983) (ME), and Edwards et al. (1988) (SM). Site abbreviations are given in Fig. 1. Flounder from Monosson and Stegeman (1991) were collected from either NR or GB. They were exposed to a single injection of 3,3′,4,4′-TCB (10 mg·kg^{-1}).

and 3,3′,4,4′-TCB are below 81.7 and 0.27 μg·g^{-1}, respectively (mean concentrations in New Bedford Harbor flounder) (Fig. 3). This strong correlation argues further for a contribution by selected PCB congeners or some covarying factor to the environmental induction.

The apparently linear correlation between CYP1A and PCB concentrations is not observed when flounder from New Bedford Harbor are included in the analysis. Elskus et al. (1989) observed that EROD activity, but not CYP1A content, was less in fish from New Bedford Harbor than in fish from two lesser contaminated sites and suggested that there was some inhibition or inactivation of CYP1A in the fish from New Bedford. Tissue concentrations of 3,3′,4,4′-TCB associated with inhibition of EROD activity in scup liver were approximately 2–3 μg·g wet weight^{-1}, although inhibition in that species may occur at tissue concentrations as low as 0.3 μg·g^{-1} (Gooch et al. 1989). The K_i for 3,3′,4,4′-TCB in scup has recently been determined to be 0.02 μM (M.E. Hahn, unpublished data). Other factors, including tissue damage or disease (Smolowitz et al. 1989; Van Veld et al. 1992) or other contaminants such as alkyltins (Fent and Stegeman 1992), could also contribute to a lower CYP1A activity in fish from highly contaminated sites. Yet, comparison of the aggregate data from the various studies (Fig. 3) supports the suggestion of inhibition by planar PCBs at New Bedford; the mechanisms and consequences of that

inhibition are not known. Structure–activity relationships for CYP1A induction by various PCB congeners and by polychlorinated dibenzodioxins and polychlorinated dibenzofurans and the possibility that some chemicals might modulate the content and/or function of the Ah receptor (e.g., Denomme et al. 1986) need to be examined further in flounder.

CYP1A Induction along the Northeast Coast

To put these data on EROD activity and CYP1A content into a larger context, we examined published values for CYP1A content or activity in flounder from eastern North America from New York along the coast to New Brunswick and Nova Scotia, going back a decade or more (Table 4; Fig. 4). The lowest values for EROD activity (0.16–0.28 nmol·min^{-1}·mg^{-1}) were seen in fish taken from offshore or from north of the most highly populated regions of New England. The highest CYP1A activities (2.0–2.4 nmol·min^{-1}·mg^{-1}) measured in flounder from sites shown in Fig. 4A are similar to those observed in Georges Bank flounder treated with 3,3′,4,4′-TCB (also shown in Fig. 4).

The CYP1A content shows a greater uniformity among most samples than does EROD activity (Fig. 4B); this uniformity is intriguing, and the reasons for it are not known. Results from selected samples stand out. These include the differences between collections at Georges Bank, the comparatively low CYP1A and EROD signal in fish from Buzzards Bay, and the high CYP1A content in Narrow River fish. Results from Narrow River and Buzzards Bay have been discussed elsewhere (Stegeman et al. 1987; Monosson and Stegeman 1991).

Georges Bank

Georges Bank can be considered remote from known PCB- and PAH-contaminated areas, and fish from there have been employed as a reference in studies of biochemical and pathological effects in flounder (e.g., Koza et al. 1993). The detection of CYP1A in Georges Bank fish is thus an important issue. Recent findings of fluorescent compounds in the bile of Georges Bank flounder (Codi 1993) are consistent with some aromatic hydrocarbon exposure. Despite the presence of CYP1A and evidence of exposure to CYP1A inducers in Georges Bank flounder, the histological features of the liver of fish there (Moore 1991; Koza et al. 1993) indicate that they are suitable reference fish for some studies. Yet, further work to assess the exposure of Georges Bank fish to CYP1A inducers is warranted.

Conclusion

This work has shown winter flounder collected from coastal sites located between Boston and New York are highly induced compared with flounder collected from offshore or from Passamaquoddy Bay. These data and other data (Monosson and Stegeman 1991; Elskus 1992) indicate that flounder at some sites may be induced to levels near the maximal capacity for response. The broad geographic distribution of induced P450 in flounder is a clear indication that inducing compounds, presumably Ah receptor agonists, are widely distributed at active concentrations. The results further indicate that PCBs probably contribute strongly to that induction. There was only a 4- to 10-fold difference in mean EROD activity between slightly and highly contaminated sites, yet the differences fit with a pattern of known sediment concentrations of PAHs and PCBs. A significant feature of our results is the demonstration that a relationship between anthropogenic contamination and induction holds true over a broad geographic range. The chronic binding of the Ah receptor by exogeneous ligands, implied by the results here, suggests that these fish may be at risk for toxicities associated with such binding (Poland and Knutson 1982). The long-term consequences of such widespread CYP1A induction and other actions of such pollutants is still uncertain. Determining the degree of induction (and other molecular changes) in extrahepatic organs could indicate the severity of chemical effects in fish from these sites.

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Larkin's Predation Model of Lake Trout (*Salvelinus namaycush*) Extinction with Harvesting and Sea Lamprey (*Petromyzon marinus*) Predation: A Qualitative Analysis

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Jensen, A.L. 1994. Larkin's predation model of lake trout (*Salvelinus namaycush*) extinction with harvesting and sea lamprey (*Petromyzon marinus*) predation: a qualitative analysis. Can. J. Fish. Aquat. Sci. 51: 942–945.

After invasion of the Great Lakes by the parasitic marine sea lamprey (*Petromyzon marinus*), lake trout (*Salvelinus namaycush*) populations crashed, but there remains some uncertainty concerning the relative importance of sea lamprey predation and harvesting in destruction of the fisheries. Some investigators believe overharvest was important; others think that the sea lamprey alone was enough. Simple models of predation assume monophagous predators and do not predict extinction of prey, but Larkin's modification of the Lotka–Volterra model results in extinction under some circumstances. The dynamics of sea lamprey predation on lake trout were investigated using Larkin's model, and crude estimates of the model parameters indicate that extinction is a likely outcome with or without a fishery.

Après l'invasion des Grands Lacs par la lamproie (*Petromyzon marinus*), un organisme parasite, les populations de touladi (*Salvelinus namaycush*), et subséquemment les pêches, se sont effondrées; mais il subsiste quelques incertitudes entourant l'importance relative de la prédation exercée par la lamproie et des pêches. Certains chercheurs pensent que la surpêche a été un facteur important tandis que d'autres pensent que la lamproie à elle seule est responsable. Les modèles simples de prédation prennent pour hypothèse que les prédateurs sont monophages et ne laissent pas prévoir l'extinction de la proie; cependant, la modification apportée par Larkin au modèle Lotka–Volterra conduit à l'extinction dans certaines circonstances. La dynamique de la prédation de la lamproie sur le touladi a été examinée au moyen du modèle de Larkin; certaines estimations grossières des paramètres du modèle indiquent que l'extinction est une issue probable, avec ou sans l'existence d'une pêche.

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The lake trout (*Salvelinus namaycush*) populations of the upper Great Lakes collapsed in the 1940s and 1950s under sea lamprey (*Petromyzon marinus*) predation and exploitation. Some investigators believe that overfishing contributed to the collapse, and some believe that sea lamprey predation alone caused the demise of lake trout. Coble et al. (1990) analyzed lake trout commercial catch and effort data and concluded that in Lakes Huron and Michigan, but not in Lake Superior, the demise of lake trout was the result of sea lamprey predation and that lake trout was not overharvested. Eschenroder (1992) disputed the analysis and the conclusions of Coble et al. (1990) and questioned their dismissal of overfishing as a cause of the lake trout decline in Lake Huron. Exploration of this issue continues to be important, as understanding the causes of lake trout decline may lead to a successful approach for lake trout recovery, and understanding of past disasters will be useful for assessment of future ecological risks.

Mathematical models have been helpful in clarifying issues and assessing the relative importance of processes involved in the demise of lake trout (e.g., Lett et al. 1975; Walters et al. 1980), but these models did not attempt to capture the feedback between lake trout and sea lamprey abundances. Lett et al. (1975) simulated the dynamics of a lake trout cohort over its lifetime at different initial lamprey densities, but initial lamprey densities was not a function of lake trout abundance, and Walters et al. (1980) assumed no feedback between lake trout and sea lamprey abundances in their simulations. The failure to develop a dynamic feedback model for sea lamprey predation on lake trout may relate to the nature of predation models. Classical predation models indicate that the result of predation is neutral stability cycles, and more recent models indicate limit cycles in which the outcome is not entirely dependent on initial conditions (e.g., Roughgarden 1979). The possibility of prey extinction as a result of predation, as occurred with sea lamprey predation on lake trout, does not appear to have been modelled mathematically, perhaps because of the prevalent historical view among ecologists that predators consume sick or old prey and have only limited effects on prey populations (e.g., Errington 1946; Lack 1954; Begon and Mortimer 1986).

The purpose of this paper is to examine a model for lamprey predation in which lake trout extinction is a possible outcome. The model was applied by Larkin (1966) for study of exploited fish populations, but he did not explore the equilibrium properties of the model.

Nearly all predation models assume monophagous predators, and yet such predators are extremely rare (Larkin 1966; Begon and Mortimer 1986). Nearly all predators, including

sea lamprey (e.g., Farmer and Beamish 1973; Farmer 1980; Smith and Tibbles 1980), consume more than one prey species. It is well known that sea lamprey destroyed lake trout populations, but at the same time sea lamprey destroyed lake trout populations, they destroyed burbot (*Lota lota*) populations and reduced lake whitefish (*Coregonus clupeaformes*) populations and populations of ciscoes (*Coregonus* spp.). Wounds from sea lamprey predation have been found on nearly all species of teleosts in the Great Lakes that have an adequate surface area for sea lamprey attachment (Farmer 1980). Sea lamprey, however, have a preference for lake trout (Farmer and Beamish 1973).

Larkin's Predation Model

To describe the relation between lake trout and sea lamprey, a model is needed in which lake trout populations will not grow indefinitely if sea lamprey are removed and in which sea lamprey do not become extinct if lake trout perish. These are the conditions that Larkin (1966) stipulated when he constructed his model for harvesting of predator and prey species. Larkin (1966) assumed that prey would be limited by other factors if the predator were extinct and that the predator would find other prey if the preferred prey became extinct. Larkin's (1966) predation model is

$$(1)\quad dN_1/dt = r_1N_1 - r_1N_1^2/K_1 - r_1a_{12}N_1N_2/K_1$$

$$(2)\quad dN_2/dt = r_2N_2 - r_2N_2^2/K_2 + r_2a_{21}N_1N_2/K_2$$

where N_1 and N_2 are abundances of lake trout and sea lamprey, r_1 and r_2 are the intrinsic rates of increase of lake trout and sea lamprey, a_{12} and a_{21} are predation coefficients (lake trout per lamprey and lamprey per lake trout), K_1 is the lake trout carrying capacity or equilibrium abundance without fishing or sea lamprey predation, K_2 is the sea lamprey carrying capacity without lake trout, and $K_2 + a_{21}N_1$ is the carrying capacity of sea lamprey with lake trout. All parameter values K_1, K_2, r_1, r_2, a_{12}, and a_{21} are greater than zero. These equations are the same as the Lotka–Volterra competition equations except for the positive sign in the last term of the second equation (e.g., Roughgarden 1979). The change of sign results in the predation model having different equilibrium behavior from the Lotka–Volterra competition model.

At equilibrium where $dN_1/dt = dN_2/dt = 0$, the relation between N_1 and N_2 is given by

$$(3)\quad N_2 = K_2 + a_{21}N_1$$

$$(4)\quad N_2 = K_1/a_{12} - N_1/a_{12}.$$

In the competition equations, with similar equilibrium relations, four outcomes between the competitors are possible (e.g., Roughgarden 1979). With the above equilibrium equations for predation, however, the slopes of equations (3) and (4) have different signs, and only two outcomes are possible. In the first case, $K_2 < K_1/a_{12}$ and the two lines cross where both N_1 and $N_2 > 0$; this intersection is a stable node with coexistence of predator and prey. In the second case, $K_2 \geq K_1/a_{12}$ and the two lines cross where $N_1 \leq 0$, and the prey becomes extinct. The preferred prey species in the first equation becomes extinct because other prey species enable the predator to maintain a sufficiently high population to exterminate the preferred prey.

Lake trout were subject both to sea lamprey predation and harvesting. Including harvesting in the equation for lake trout gives

$$(5)\quad dN_1/dt = r_1N_1 - r_1N_1^2/K_1 - r_1a_{12}N_1N_2/K_1 - qEN_1$$

where the additional terms are a catchability coefficient q and fishing effort E. The equations for equilibrium now become

$$(6)\quad N_2 = K_1\,(1 - qE)/a_{12} - N_1/a_{12}$$

$$(7)\quad N_2 = K_2 + a_{21}N_1$$

and the condition for coexistence of lake trout and sea lamprey becomes

$$(8)\quad K_1\,(1 - qE)/a_{12} > K_2.$$

The above equation indicates that potentially every level of fishing has some effect on the outcome of sea lamprey predation on lake trout. To assess the effect of overfishing on the destruction of lake trout, I applied the above equations using data for Lake Michigan.

Results and Discussion

Parameter estimation for models of interacting populations is difficult, but only crude estimates are necessary to determine whether extinction of lake trout under sea lamprey predation is likely. The maximum historical harvest of lake trout from Lake Michigan was about 4 million kg (Farmer and Beamish 1973), and a reasonable first estimate of the lake trout carrying capacity K_1 is 4 times the maximum yield (Pella and Tomlinson 1969) or 16 million kg. Assuming that each lake trout in the recruited population weighed about 0.80 kg (Walters et al. 1980) results in about 20 million lake trout at the carrying capacity. Lett et al. (1975) studied the effect of sea lamprey on lake trout populations and their data can be applied to estimate a_{12}. Lett et al. (1975) gave two equilibrium levels of lake trout and sea lamprey (N_1,N_2) as (200,17) and (10,50); substitution of these values into equation (4) gives two equations in two unknowns and $a_{12} = 5.75$. If lake trout were exploited at near the MSY, the abundance of lake trout without sea lamprey predation would be $K_1/2$, and extinction as a result of sea lamprey predation would occur when $K_2 \geq 1.7$ million sea lamprey (equation (8)); without exploitation, extinction will occur when $K_2 \geq 3.4$ million sea lamprey.

Although the parameter estimates are crude, substantial changes in the parameter estimates still result in extinction of lake trout with a sea lamprey carrying capacity without lake trout, K_2, of a few million, and a sea lamprey carrying capacity of a few million supported by predation on burbot, lake whitefish, and species other than lake trout is likely. Extinction of the lake trout appears a reasonable outcome with or without fishing. Fishing reduces the abundance of lake trout and enables the sea lamprey to more easily exterminate them, but this would be of practical significance only when the carrying capacity of sea lamprey without lake trout was not high enough to exterminate an unexploited lake trout population. The likelihood of extinction increases with the biomass of secondary species able to support the sea lamprey population, which varies from lake to lake. Lakes Michigan and Huron are more productive than Lake Superior, indicating that the sea lamprey's relative carrying capacity without lake trout would be higher in Lakes Michigan and Huron, and this would increase the likelihood of lake trout extinction in these lakes. Fishing

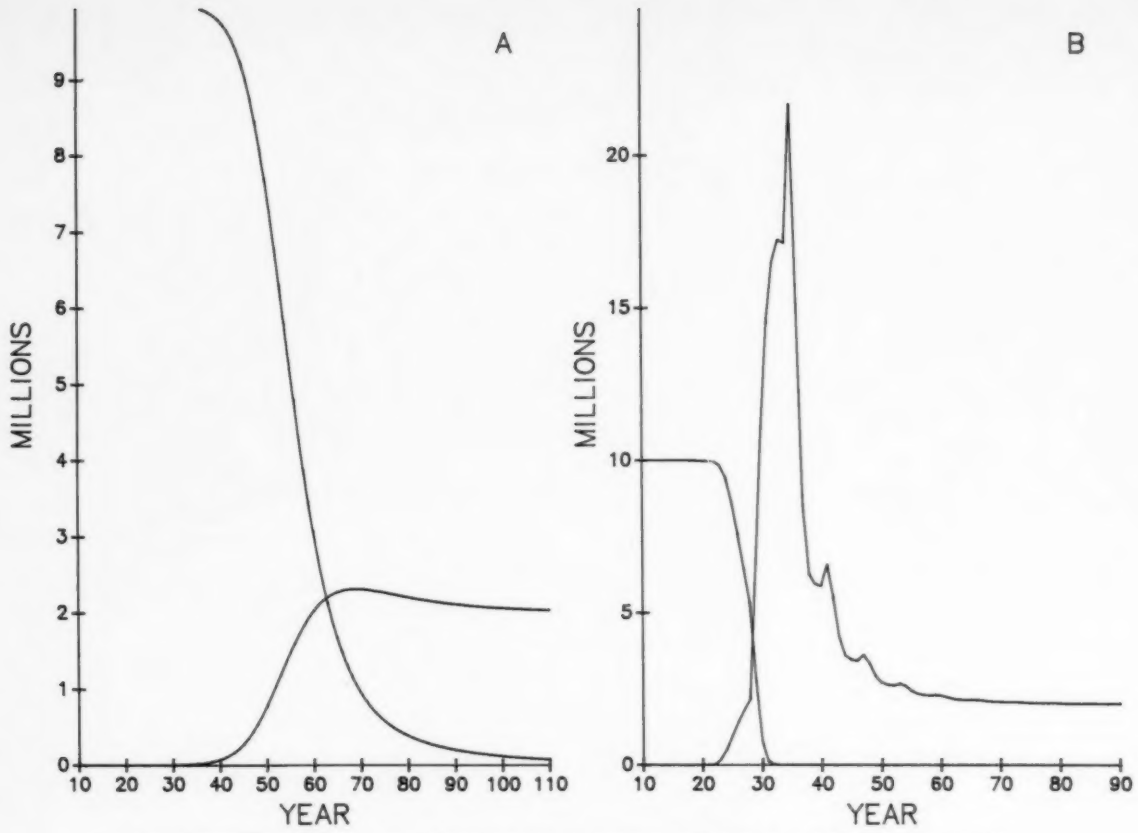

FIG. 1. Simulated abundances of lake trout and sea lamprey with (A) $a_{21} = 0.20$ and (B) $a_{21} = 20$.

might have been of practical importance only in Lake Superior, and this is where Jensen (1978) and Coble et al. (1990) observed overharvesting.

Larkin's model was applied to simulate lake trout and sea lamprey dynamics to determine if the observed pattern could be reproduced. For this purpose, an estimate of a_{21}, the number of lamprey produced per lake trout, is necessary. Field data for this estimate are not available, but some indication of possible values can be obtained from fecundity and mortality estimates. At equilibrium, sea lamprey would replace themselves, and studies indicate that each lamprey would kill four or five lake trout (Lett et al. 1975); the minimum possible value of a_{21} is therefore about 0.20. The maximum possible value of a_{21} is large. Each female feeds on four or five lake trout and produces 50 000 to 70 000 eggs of which about 95% are spawned, and of these, about 14% remain in the nests (Potter 1980). About 88% of the eggs in the nest survive and hatch, and although mortality immediately after leaving the nest is high, it decreases later in life (Potter 1980). The percent female varies; it was less than 30% during periods of rapid population growth. The above estimates indicate that a_{21} could be large even if only a small proportion of eggs survived to adulthood. Rather than attempt a more precise estimate of a_{21}, I did simulations with a_{21} between 0.20 and 20; these values describe the possible patterns of sea lamprey population growth obtainable with Larkin's model.

Other parameters used in the simulations were $F = 0.25$ (value for MSY), $K_2 = 2$ million sea lamprey, $K_1 = 20$ million lake trout, $r_1 = 0.50$, and $r_2 = 0.75$. To obtain a more realistic simulation, a reproductive time lag of 6 yr was applied because the sea lamprey larval stage lasts from 6 to 8 yr and larvae do not prey on lake trout (Potter 1980). The equation for simulation of sea lamprey abundances was

$$dN_2/dt = r_2N_2\,(t-6) - r_2N_2(t)^2/K_2 + r_2a_{21}\,N_1\,(t-6)\,N_2\,(t-6)/K_2$$

where all terms have been defined above. A reproductive time lag does not cause cycles in abundance like the time lag introduced by Hutchinson (1948), but a reproductive time lag does delay the increase in lamprey abundance. The equations were solved using Euler's method with a time interval of 0.01 yr. The initial values were 10 million lake trout and 10 sea lamprey.

The pattern of sea lamprey population growth was determined by the value of a_{21} (Fig. 1). With a_{21} at 0.20 lamprey per lake trout, there is only a small peak in abundance of sea lamprey, and lamprey population growth is nearly asymptotic; with a_{21} at 20 lamprey per lake trout, the peak sea lamprey abundance was large and there were small fluctuations that resulted from the time lag in reproduction; this last curve is similar to those observed for the Great Lakes (e.g., Smith and Tibbles 1980). The simulations were done with additional values of a_{21} to determine when the sea lam-

prey growth curve begins to become similar to that observed for the Great Lakes, and with a_{12} equal to just 1, there is a peak in lamprey abundance of 3.9 million before numbers decrease back to the carrying capacity of 2 million. Larkin's model does describe the observed pattern of sea lamprey and lake trout abundances.

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Placing Odds on Sustainable Catch Using Virtual Population Analysis and Survey Data

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Virtual population analysis (VPA) is often used in conjunction with survey data to provide estimates of current stock size and an allowable catch that is sustainable in the sense that it is expected to produce some desired trend (or stability) in stock size. But these estimates are generally very uncertain, and should be accompanied by statements about the odds of sustainability for a range of allowable catch options. The odds can be expressed as an easily understood short-term risk function, but potential cumulative risks associated with the function should be simulated as a long-term guide for management. Bayesian estimates of the odds of sustainability can be calculated using the likelihood function of the survey data for different current stock size and natural mortality rate possibilities, with estimates of expected recruitment and recruitment variability. Prerecruitment year classes in the survey are included in the calculation of expected recruitment and its uncertainty, allowing use of survey data from gears with different (arbitrary) age selectivity from the main fishery gear. Application of the calculations to a yellow perch (*Perca flavescens*) fishery illustrates how critical it is to have a well-designed survey, even when much age composition data for VPA are also available.

L'analyse virtuelle des populations (VPA) est souvent associée aux résultats de dénombrements pour produire des estimations de l'importance des stocks actuels ainsi que de la prise admissible à un niveau soutenable, au sens où on s'attend qu'elle produira une tendance souhaitée (ou la stabilité) chez ce stock. Mais ces estimations sont généralement très incertaines et devraient être accompagnées d'explications relatives aux probabilités de la soutenabilité d'une série de niveaux de prises admissibles. Ces probabilités peuvent être exprimées au moyen d'une fonction de risque à court terme qui est de compréhension facile, mais il faudrait alors que les risques cumulatifs potentiels qui sont associés à la fonction fassent l'objet d'une simulation qui prendrait la forme de valeurs de référence à long terme pour la gestion. Les estimations bayésiennes des probabilités de la soutenabilité peuvent être calculées au moyen de la fonction de probabilité d'apparition des données des dénombrements en fonction de différents niveaux de stocks actuels et des possibilités d'apparition des taux de mortalité naturelle, et au moyen des estimations du recrutement attendu et de la variabilité de celui-ci. Les classes d'âge qui précèdent le recrutement, dans le dénombrement, doivent figurer dans le calcul du recrutement attendu et de l'incertitude qui s'y rattache, compte tenu des données de dénombrement obtenues à partir d'engins dont la sélectivité (arbitraire) par groupe d'âge diffère de celle des engins qui servent à la pêche principale. L'application de ces calculs à la pêche à la perchaude (*Perca flavescens*) montre à quel point il est crucial de pouvoir tabler sur un dénombrement bien conçu, même lorsqu'on possède beaucoup de données sur la composition par groupe d'âge en vue de la VPA.

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With the development of risk assessment procedures, fisheries scientists have started to present assessment advice in probabilistic terms that reveal much more clearly just how uncertain we are about sustainable harvest levels in many fisheries even when masses of age composition and survey data are available (Bergh and Butterworth 1987; Nakamura et al. 1989; Francis 1992; Restrepo et al. 1992; Rosenberg et al. 1992). This development is much needed; too often, we have seen major social disasters within a decade or two after fisheries development policies were initiated with at least some guidance from assessments that turned out to be overly optimistic. Perhaps there would still be a healthy anchoveta fishery off Peru had it been more forcefully pointed out in the early 1960's that there was a good chance of not sustaining catches as high as early catch-effort assessments indicated; perhaps a large proportion of Newfoundland's fishermen would not be unemployed today if it had been stressed more strongly in the late 1970's that there was a substantial risk that our northern cod stock would not rebuild if we even partially replaced foreign fleets with our own offshore trawlers.

While it is easy to see that assessment advice should be couched in probabilistic terms (e.g., "the odds of sustaining catch X are so-and-so" or "the odds of causing a collapse under policy Y are so-and-so"), there is as yet no concensus about how to do the probabilistic calculations so as

to fully reflect important uncertainties, and how to present the results in an understandable format for management. Recommendations for calculation range from treating the sampling distribution of point estimates from virtual population analysis (VPA) as an indication of uncertainty (Restrepo et al. 1992) to Bayesian methods (Nakamura et al. 1989; Walters and Ludwig 1994) and related calculations based on use of confidence distributions (Francis 1992). To the extent that probabilistic statements about the effect of policy options depend on assigning probabilities to unknown population parameters (or "states of nature"), it has been argued that only Bayesian methods are strictly appropriate; other statistical approaches do not even admit that it is valid to assign probabilities to parameter values (Walters and Ludwig 1994). Recommendations for presentation of results range from providing point estimates and conservative confidence limits to providing risk functions representing the total odds of success (or failure) for a range of policy choices.

There has been a strong move toward presenting results in terms of risk functions, especially by scientists who have enough experience in actual management deliberations to have seen how common it is for various management actors to completely misinterpret point estimates and limits. The move comes none too soon; much scientific advice has been couched in complex presentations that appear to have been aimed more at *impressing* decision makers with the thoroughness of analysis than with *informing* them about risks associated with trusting it.

This paper develops a Bayesian approach to the analysis of catch-at-age and survey data, with the aim of providing a simple risk function for use in management situations where annual deliberations are used to set an allowable catch limit. We begin by suggesting how to construct a single risk function that is easily understood by various management actors, and involves separation of short-term risk considerations from long-term management objectives related to harvest development or stock rebuilding. We then show one method to calculate this function using the likelihood function for the survey data given numbers-at-age estimates from VPA, along with estimates of variability in stock size and recruitment as evidenced in the VPA results. Finally, we apply the method to an example, yellow perch (*Perca flavescens*), in Green Bay, Lake Michigan, that illustrates the critical importance of survey data.

A Risk Function for Sustainable Harvest Management

Various risk functions have been proposed to convey information about scientific uncertainty to fishery managers, from simple graphs showing ranges of possible change in stock size for different catch options (Restrepo et al. 1992) to plots of probabilities of stock collapse within some specified number of years for alternative quotas or quota reduction policies if applied over several years (Francis 1992). These options reflect how managers faced with the task of recommending what to do this year are actually facing two quite distinct questions: (1) what are the possible immediate consequences of this year's decision (what will the stock size be next year)? and (2) what long-term pattern of change in stock size is most desirable and attainable (what legacy should be left for decision makers next year and into the future)? Fisheries management theory has mainly been developed to help deal with the second question, through assessment of maximum sustainable field (MSY), optimum stock size, and feedback/adaptive policies for maximizing various measures of long-term fishery performance.

But for making a decision *this year*, the results of long-term analyses can be expressed as a target stock size T_t to be left after this year's harvest. This target can be measured as a growth factor G times last year's stock size S_{t-1}. Then attempting to maintain a stable stock size is expressed by setting $T_t = S_{t-1}$; attempting to rebuild stock size toward some long-term higher level is represented by $T_t = GS_{t-1}$ where $G > 1.0$ defines some socially and economically acceptable rate of stock rebuilding; and allowing the stock size to decline toward some presumably more productive level is represented again by $T_t = GS_{t-1}$ but with $G < 1.0$ defining an acceptable rate of depletion. Note that debate about long-term objectives and acceptable values of G may involve questions of uncertainty (e.g., what is a "safe" $G < 1.0$ during fishery development, or should a $G < > 1.0$ be chosen to deliberately experiment with stock size to empirically find an optimum long-term target). However, this debate can proceed even without such questions and should not be confused with debates about how big a chance (of not achieving T_t) to take this year.

Couching discussion about long-term management objectives in terms of the "G convention" as defined above has an important implication for the presentation of probabilistic assessments. Suppose we define an allowable catch option C_t to be "sustainable" if it results in the simple short-term condition $S_t/S_{t-1} \geq G$. Suppose then that we assign probabilities to every possible combination S_{t-1}, S_t based on the data, i.e., we admit that neither last year's stock size (S_{t-1}) nor this year's stock after harvesting (S_t) is known (or ever will be known) with any certainty. Then the probability that C_t is sustainable is just the sum or integral of all those S_{t-1}, S_t probabilities that meet the G condition. Computing this sum for a range of C_t options is trivial and leads to a management risk function (Fig. 1) that is very easy to understand and interpret.

Further, the risk function in Fig. 1 is presented as a positive statement about the chances of sustaining various catch levels (meeting G objective), rather than a negative statement or warning about the odds of something bad happening. Although this is obviously only a ploy for presentation (one could as easily plot 1 minus the odds of sustaining each catch and call it a risk of not meeting the long-term objective), it is one that can make a big difference to the way decision makers react to the risk information. Too often, fisheries scientists step beyond the bounds of their competence or responsibility by offering highly conservative and risk averse advice (Pearse and Walters 1992). This advocacy role makes their advice and analyses immediately suspect, especially when the advice is couched in terms of negative outcomes or warnings. While the format of presentation in Fig. 1 will not eliminate such suspicions, it will at least narrow any debate about scientific objectivity to the statistical question of whether the data justify assigning a narrower distribution of outcomes than scientists believe can be justified on the basis of their analyses.

Questions about long-term objectives (choice of G) and short-term risk taking (Fig. 1) become entangled when decision makers ask questions of the form "how will it affect our chances of meeting the long-term objective if we and

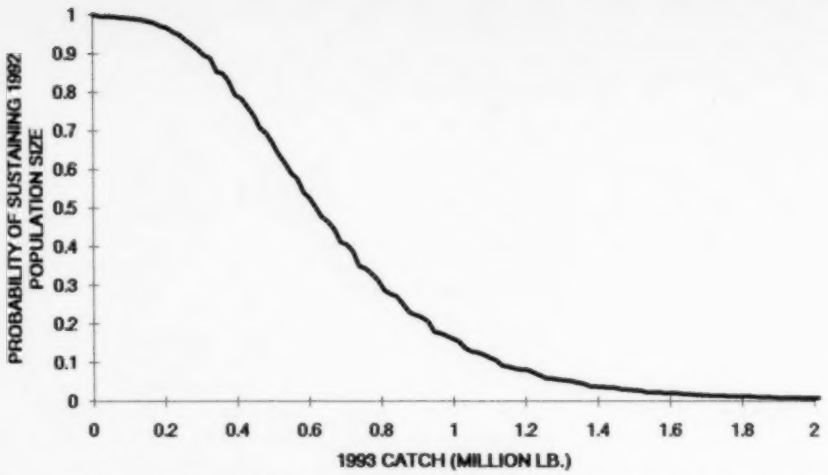

FIG. 1. Risk function representing the probability that stock size after harvest this year will be at least as large as the stock size after harvest last year (target population growth factor $G = 1$) for alternative allowable catches this year. Example values are for the yellow perch fishery of southern Green Bay, Lake Michigan; catch reported in pounds, where 1 lb ≈ 0.4 kg.

our successors choose a catch each year that has an $X\%$ chance of not meeting the objective for that year?". Here the scientist must be very careful because what the decision makers are presumably seeking is some simple formula or standard for setting an acceptable level of annual risk. In response, the scientist can (and presumably should) undertake the task of setting up Monte Carlo simulations and/or a management simulation game to calculate the spread or distribution of long-term trajectories that might result from any choice of X, as for example in Pelletier and Laurec (1992). The scientist's answer to the decision makers will then be something like "If you allow a 50% risk every year, then there is a 5% chance that you will still be below the long-term objective after 20 yr; but if you allow only a 20% annual risk, then there is less than a 1% chance of still being below target then." What scientists must not do at this point is to allow the decision makers to pass the burden of judgement to them, by trying to answer the inevitable follow-up question "but is 20 yr long enough, and is a 5% risk acceptable?" For answers to such social and economic questions, decision makers should be turning not to scientists but rather to the people that they represent (especially those whose lives and livelihoods depend on the fishery).

Constructing Probability Distributions for Stock Size Using VPA

For the following development, assume that the basic data for assessment consist of two matrices, C and D: c_{at} are catches at age ($a = 1...n$) over time ($t = 1...T$) and d_{at} are survey-based estimates of relative abundance at age ($a = 0...n$ where 0 allows for a prerecruit component in the survey). Here, age is measured relative to the first biological age caught ($a = 1$), and T refers to last year. For VPA, another key input to analysis is the natural mortality rate M. To construct the risk function of Fig. 1, we seek to analyse the data so as to assign Bayes posterior probabilities $p(S_T,M|D)$ to each possible stock size S_T that might have been left at the end of last year as a result of C_T and of natural mortality rate M, and conditional probabilities $p(S_{T+1}|S_T,M,C_{T+1})$ for each possible stock size S_{T+1} that may arise as a result of allowable catch decisions C_{T+1} this year. Following the approach suggested in Walters and Ludwig (1994), $p(S_T,M|D)$ will be computed below as the marginal likelihood of D given S_T and M, divided by the sum of such likelihoods over all values of S_T and M admitted to have nonzero prior probability of being correct. Various approaches can then be used to estimate $p(S_{T+1}|S_T,M,C_{T+1})$.

Computation of the likelihood of D given S_T,M proceeds in two steps. First, S_T is used to initialize the VPA "backward simulation" of a historical numbers at age matrix N_{at}, treating the catch data C and M as fixed, known inputs to the simulation. Then the likelihood of D is expressed as a function of N.

Calculation of Historical Numbers at Age Given S_T,M

To simplify the following presentation, assume that the fishery takes place over a short season each year, so that individual cohorts in the stock decline in numbers N according to the survival model $N_{a+1,t+1} = (N_{a,t} - c_{a,t})s$ where $s = e^{-M}$ is an annual survival rate (M = instantaneous natural mortality rate). Assume further that essentially no fish are left alive after age n ($N_{n+1,t} = 0$). Similar derivations apply for the continuous fishery case and cases where significant numbers of fish are still alive at age n, although the more complex equations involved seldom matter much to the basic predictions. The seasonal catch can be expressed in terms of age–time specific exploitation rates u_{at} as $c_{at} = u_{at}N_{at}$. As is usual for VPA, assume that the u_{at} meet the following regularity (or separability) conditions for the last few (T_r) years ($t = T - T_r...T$): (1) $u_{at} = v_a u_t$ where v_a is the relative vulnerability (or proportion of fish vulnerable) of age a fish and u_t is an overall exploitation rate for year t and (2) $u_{at} = u_t$ for $a_1 \le a \le a_2$, where a_1 and a_2 represent a range of ages of "full vulnerability" to the fishery. For most fisheries, selectivity patterns change substantially over time due to changes in gear, growth, and spatial distribution of fishing effort; thus the averaging "window" $T - T_r, a_1 - a_2$

should be restricted to a few years and ages. Under these assumptions, the backward simulation of $N_{a,t}$ is trivial for $t < T$ and $a \leq n$:

$$(1) \qquad N_{a,t} = c_{at} + N_{a+1,t+1}/s$$

and the only serious issue is how to initialize the calculations with terminal numbers N_{aT}. Here the usual VPA approach would be to compute N_{aT} from the terminal exploitation rate $u_T = C_T/(C_T + S_T)$ where

$$C_T = \sum_{a=1}^{n} c_{aT}$$

and vulnerabilities v_a as

$$(2) \qquad N^*_{a,T} = c_{a,T}/(v_a u_T).$$

Note that this parameterization initializes the backward calculation in terms of S_T rather than a "terminal fishing rate" or "terminal F". Estimates of the v_a can come from outside the analysis, but would ordinarily be obtained by "iterating" the VPA for each S_T,M: repeat the eq. (1) and (2) calculation a few times, each time taking v_a to be the averages of v_{at} over the t window $T - r \ldots T - 1$ from the last iteration as the estimates of v_a. (As an aside, iteration can often be avoided by doing the back-calculation and averaging in a particular order: first work backward in time over only fully vulnerable ages and then downward in age over the partially recruited ages.)

However, the initialization represented by eq. (2) does not make full use of the data and gives estimates of N_{aT} that have high variance especially for young ages where $v_a u_T$ is small. If the survey method selectively targets younger fish, then the survey estimator $N^{**}_{aT} = d_{aT}/q_a$ where q_a is the estimated survey catchability coefficient for age a fish (see below) may be more reliable, especially when it comes time to assess $p(S_{T+1}|S_T,M,C_{T+1})$ later in the analysis. The estimates N^*_{aT} and N^{**}_{aT} can be combined to give a weighted, approximate maximum likelihood estimator (conditional on S_T, M):

$$(3) \qquad N_{aT} = g_a N^*_{aT} + (1 - g_a) N^{**}_{aT}$$

where the weights $g_a = \mathrm{var}(N^{**}_{aT})/[\mathrm{var}(N^*_{aT}) + \mathrm{var}(N^{**}_{aT})]$ are computed from the variance approximations

$$(4) \qquad \mathrm{var}(N^*_{aT}) = C_T^2 p_a(1 - p_a)/n_a(v_a u_T)^{-2}$$

$$(5) \qquad \mathrm{var}(N^{**}_{aT}) = \sum_{t=1}^{T-1} (N_{at} - d_{at}/q_a)^2/(T-1)$$

where p_a is the proportion of age a fish in the year T catch ageing sample and n_a is the size of that ageing sample. Note that eq. (5) uses the differences between VPA estimates of N_{at} and survey-based estimates (d_{at}/q_a) as an "empirical" measure of the accuracy of the survey estimates for earlier years; for this variance estimate and the associated weighted estimates of eq. (3) to converge, it is necessary to iterate the whole VPA calculation a few times; this can be done in the same iteration "loop" as is used for the v_a estimation (the resulting iteration scheme is similar to the "shrinkage" method suggested by Rosenberg et al. 1992). Other, more complex weighting and iteration schemes can be constructed using nonlinear estimation in conjunction with the likelihood function for D, following procedures such as used in the ADAPT system (Gavaris 1988).

Likelihood of the Survey Data Given S_T,M

Assume that the survey estimates d_{at} of relative abundance are on average proportional to the number of fish N'_{at} alive at some survey time each year and are measured with multiplicative, log-normal errors w_{at}, so that

$$(6) \qquad d_{at} = q_a N'_{at} \exp(w_{at}).$$

The survey catchabilities q_a may vary arbitrarily with age a. The q_a values near 1.0 would represent survey data that have been expanded to total population estimates via some swept area or related calculation; however, in the derivation below, it will not be assumed that any such expansion has been carried out successfully, so q_a will be allowed to take any positive value with equal prior probability. The N'_{at} may be just N_{at} for preharvest survey systems, or $N_{at} - c_{at}$ for postharvest surveys, or $(N_{at} - c_{at})s'$ for surveys taken after a period over which survival s' has taken place following the harvest. Assume further that the errors w_{at} have two components: $w_{at} = w_t + w'_{at}$ where w_t represents a survey "year effect" (independent between years) and the w'_{at} represent independent age–time effects due to varying spatial distributions, ageing errors, etc. That is, the errors are correlated across ages within years (typical in fisheries data sets). Note that the w_{at} represent a complex of errors arising from both the survey process and the VPA calculation of N'_{at}; for a recent review of literature about error sources related to VPA, see LaPointe et al. (1992). Given predictions N'_{at} computed as a function of S_T and M (i.e., conditional on S_T and M), and given particular values for the q_a, the likelihood function for the data d_{at} is found from the logarithm of eq. (6). Let

$$(7) \qquad z_{at} = \log(d_{at}/N'_{at}) = q'_a + w_{at}$$

where $q'_a = \log(q_a)$. Then, each time-dependent normal error w_t is given by

$$(8) \qquad w_t = \sum_{a=1}^{n} (z_{at} - q'_a)/n$$

and the age–time dependent normal errors are given by

$$(9) \qquad w'_{at} = z_{at} - q'_a - w_t.$$

Then the likelihood of the data given S_T,M and the q'_a can be expressed in terms of these normal errors and is just proportional to the exponential "kernel" L given by (see Walters and Ludwig 1994):

$$(10) \qquad L = \exp\left[-\sum_{t=1}^{T} w_t^2/(2V_1) - \sum_{t=1}^{T}\sum_{a=1}^{n} w_{at}^2/(2V_2) \right]$$

where V_1 is the time variance in the survey estimates and V_2 is the age–time variance. Using the results in Walters and Ludwig (1994), integration of eq. (10) over the "nuisance" parameters V_1, V_2 and the q'_a (assuming uniform prior probabilities for the q'_a and prior probabilities for the V's proportional to $1/V$) gives the marginal likelihood kernel

$$(11) \qquad L^{q,V} = \left[\mathrm{SS}_t \prod_{a=1}^{n} \mathrm{SS}_a \right]^{-(T-1)/2}$$

where the sums of squares SS are given by

$$\mathrm{SS}_t = \sum_{t=1}^{n} w_t^2$$

and

$$SS_a = \sum_{t=1}^{n} w_{at}^2$$

computed using eq. (8) and (9) with q_a' replaced by the conditional (on S_T,M) maximum likelihood estimators:

$$(12) \quad q_a' = \sum_{t=1}^{T} z_{at} / T.$$

Note that the conditional maximum likelihood estimators of q_a, needed for the iterative calculation of N_{at} proposed in eq. (3)–(5), are given by $\exp(q_a')$. Note also that eq. (11) is an approximation even if the assumption of log-normality of residuals is correct, since it does not involve integration over nuisance parameters (N_{aT}, v_a) that appear in the dynamic model prediction of N_{at} given S_T,M; justification for using the conditional maximum likelihood estimators of these nuisance parameters, rather than integration over them, is provided in the Appendix.

Calculation of the Posterior Probability $p(S_T,M|D)$

The Bayes posterior probability $p(S_T,M|D)$ for any S_T,M combination is then given by the L^{qV} value for that combination calculated from eq. (10), divided by the sum of the L^{qV} values calculated for all S_T,M combinations admitted to have nonzero prior probability. If a nonuniform prior probability distribution appears warranted for M, then the posterior probability becomes L^{qV} multiplied by this prior probability, divided by the sum of such products (i.e., just replace L^{qV} by $L^{qV}p(M)$ in the ratio calculation where $p(M)$ is the prior probability for M).

The above procedure for calculating the likelihood function and posterior distribution does not depend in any way on the particular method used to back-calculate N_{at}' given S_T,M. Thus it can be used with any VPA or catch-at-age procedure, such as ADAPT (Gavaris 1988), the separable VPA method (Pope and Shepherd 1982), or CAGEAN (Deriso et al. 1985). All that is required is to provide the procedure with a "front end" for (1) expressing the terminal stock size calculation (N_{aT}) in terms of S_T rather than the usual terminal fishing rate parameterization and (2) repeating the procedure and the likelihood calculation over a grid of S_T,M combinations. In practice, this grid need not even be very fine. Repeating the procedure at 30 S_T × 5 M levels (150 runs of the procedure) is usually enough to provide a quite adequate "picture" of the posterior distribution for further risk analysis. Note that this represents a considerable savings in computation compared with the Monte Carlo approach suggested by Restrepo et al. (1992), but at the expense of not accounting completely for the effect of uncertainty in the c_{at} data on the posterior distribution (see Appendix).

Maximization of L (or L^{qV}) with respect to S_T would be what some authors have called a "tuning procedure" for VPA, i.e., finding the S_T (or equivalently, terminal F) value that makes the survey data appear most likely (gives N_{at}' that best fit trends in survey data D). However, this point estimator is not needed at all for the risk analysis. Further, the term "tuning" connotes to many people a process of making minor corrections or adjustments to parameters, and should therefore be avoided; commonly, the posterior distribution (or confidence limits) for S_T is quite broad (a wide range of S_T values can almost equally well explain typical trends in survey data). Usually the only way to avoid such a broad distribution is to make the pretense that the q_a are known in advance (i.e., that the survey provides unbiased estimates of absolute stock size), which is almost always an overly optimistic assumption.

Note that the error structure assumed in eq. (7)–(11) is not strictly correct unless the catches at age c_{at} have been measured exactly and M is truly constant over age and time. Measurement errors in the c_{at} create correlated errors over age within each cohort in the VPA calculation of N_{at}'. These errors appear in the calculation of w_{at} (i.e., are components of w_{at} along with random survey errors and errors in model structure due to nonconstant M), and hence will contribute to making the posterior distribution for S_T,M broader than it would otherwise be. But the calculation of L^{qV} ignores the within-cohort correlation structure, and including this structure in the calculation could in principle lead to even higher uncertainty (even broader posterior). It is not yet known how to include such complex correlation structure in the computation of marginal likelihood kernels, without massive computational effort.

Uncertainty in the Prediction of this Year's Stock Given S_T

There are at least three alternative approaches to assessment of the other probability distribution, $p(S_{T+1}|S_T,M,C_{T+1})$, needed for short-term risk assessment. The analysis begins by noting that for seasonal fisheries, it is sufficient to find a probability distribution for the preharvest biomass $B_{T+1} = S_{T+1} + C_{T+1}$. Then for each possible S_T,B_{T+1} combination that is assigned some nonzero probability, it is easy to find the largest C_{T+1} that will meet the short-term sustainability criterion ($B_{T+1} - C_{T+1} > GS_T$) and accumulate probabilities for narrow ranges or "bins" of such C values to produce graphs like Fig. 1 (the sum of accumulated probabilities for C bins representing $C \leq C^*$ is the probability that a catch of at least C^* will be sustainable). Options for estimating $p(B_{T+1}|S_T,M,D)$ generally involve assuming some functional form for p (e.g., normal or lognormal) and then estimating the variance of this distribution by (1) using analytical approximations based on uncertainty in the variables used to predict B_{T+1}, (2) using a Monte Carlo simulation procedure such as suggested by Restrepo et al. (1992), or (3) using an empirical "past prediction performance" (PPP) procedure based on the historical prediction performance of the VPA. The first two of these options have been well reviewed and described by Restrepo et al. (1992) and will not be discussed further here. A PPP procedure is developed below, which should generally give very conservative (high) variance estimates compared with the other alternatives; any proposal to use a less conservative alternative needs to be justified by explaining why its lower variance should be trusted considering the historical variance in predictions as evidenced in the PPP estimator.

Prediction of B_{T+1} from the VPA abundance estimates involves two component predictions: new recruits and post-recruit survival:

$$(13) \quad B_{T+1} = v_1 N_{1,T+1} W_1 + \sum_{a=1}^{n-1} (N_{aT} - c_{aT}) s v_{a+1} W_{a+1}.$$

Here, new recruits are represented by $N_{1,T+1}$, and the W_a

represent body-weights-at-age if the prediction is to be made in terms of stock biomass. Note that the N's and v's used in this calculation are best estimates conditional on S_T,M; hence, errors in these estimates will contribute to variance of the B_{T+1} prediction, along with natural variation in growth rates, s (e^{-M}), and $N_{1,T+1}$. Considering this smorgasbord of errors, it is obviously unwise to assume a low value for the prediction variance.

But on the positive side, note that variation in new recruits ($N_{1,T+1}$) does not always represent a major component of uncertainty in the B_{T+1} prediction, especially if v_1 or W_1 is small. By using age composition data, the VPA "tracks" recruitment variation as cohorts pass through partially vulnerable ages, hence helping to reduce short-term prediction error. This note of optimism also emphasizes the fundamental distinction between short-term recruitment forecasting versus understanding enough about the long-term (average) relationship between stock and recruitment to make intelligent choices about development strategies (choices of G). In seeking ways to reduce the variance of B_{T+1} by finding a low-variance predictor of $N_{1,T+1}$, about the last place to look is in the long-term stock–recruit relationship. Generally, far more precise predictors can be obtained from (1) prerecruit survey components ($N_{0,T}$), (2) time series models that capture autoregressive features in the $N_{1,t}$ historical estimates, and (3) environmental factor–recruitment correlation analysis again using the $N_{1,t}$ history. Combinations of such predictors can be developed using weighting schemes such as in eq. (3)–(5).

A simple prerecruit survey predictor for $N_{1,T+1}$ in eq. (13) can be obtained by assuming density-independent survival rate s_0 from age 0 to 1 (not necessarily a good assumption; see example below), i.e., $N_{1,t+1} = s_0 N_{0t}$. If the survey observation for N_{0t} is assumed to be $d_{0t} = q_0 N_{0t} \exp(w_{0t})$, w_{0t} normal, then the conditional (on S_T,M) maximum likelihood predictor $N^*_{1,T+1}$ of $N_{1,T+1}$ from d_{0T} is given by

$$(14) \quad N^*_{1,T+1} = d_{0T} \exp(-q'_0)$$

where the conditional maximum likelihood estimator of $q'_0 = \log(q_0/s_0)$ is just

$$(15) \quad q'_0 = \sum_{t=1}^{T-1} \log(d_{0t} / N_{1,t+1}) / (T-1).$$

Equations (14) and (15) amount to finding the 0 intercept linear regression relationship between d_{0t} and $N_{1,t+1}$ evidenced by the VPA results while assuming log-normal errors in the measurement and survival process. Note that this calculation is in the same format as used for q'_a in the likelihood assessment above and can be done at the same time if it is reasonable to assume that w_{0t} consists of the same w_t and w'_{0t} components as the rest of the survey data (i.e., prerecruits collected by same survey samples as older fish).

Given a method for predicting B_{T+1} using eq. (13) in conjunction with whatever method is considered most appropriate for predicting $N_{1,T+1}$, a conservative PPP estimator for the variance of B_{T+1} can be constructed as follows. First, calculate "best" available estimates B^*_t for the historical preharvest biomasses using the final VPA results (data for $t = 1 \ldots T$, conditional on S_T,M):

$$(16) \quad B^*_t = \sum_{a=1}^{n} N_{at} v_a W_a .$$

These estimates are presumably most reliable for earlier years ($t < T - n$) for which the N_{at} estimates involve only completed cohorts. Next, treat each year $t < T$ as though it were the terminal year and calculate B^{**}_{T+1} as

$$(17) \quad B^{**}_{t+1} = v_1 N^*_{1,t+1} W_1 + \sum_{a=1}^{n-1} (N^{***}_{at} - c_{at}) s v_{a+1} W_{a+1}$$

where N^{***}_{at} is calculated from the terminal numbers eq. (3) but using the v_a,q_a,u_T, and g_a available at time T in calculating the estimators N^*_{at} and N^{**}_{at}. The B^{**}_{t+1} is just the eq. (13) predictor of B_{t+1} that would have been made at time t if the current (time T) parameter estimates had been available then; note that one source of error in this predictor is change in vulnerability, exploitation rate, and body size over time — not accounting for such changes is one factor that makes the following variance calculation very conservative. Finally, the PPP variance calculation is quite simple:

$$(18) \quad \mathrm{var}(B_{T+1}) = \sum_{t=2}^{T-k} (B^{**}_t - B^*_t)^2 / (T-k-2)$$

where $T - k$ is the last year for which the B^*_t are considered to be reliable (based on mostly completed cohorts). If it is assumed that B_{T+1} is log-normally distributed, replace B^{**}_t and B^*_t with their logarithms before performing the eq. (18) calculation. Equations (16) and (17) may well lead to highly patterned residuals $B^{**}_t - B^*_t$ over time, indicative of historical changes in vulnerability, growth, and exploitation rate. If examination of these residuals suggests that the variance estimator from eq. (18) is unreasonably high, alternative estimators can be obtained by (1) using only more recent years in the eq. (18) sum or (2) replacing each B^{**}_t by the predictor obtained from iterated VPA using only $t - 1$ years of data and a terminal u_{t-1} more appropriate for time $t - 1$ (e.g., use $u_{t-1} = C_{t-1}/B^*_{t-1}$). However, any such optimistic estimators will require careful justification because they may result in substantial underestimates of the risk of not meeting the G goal for high C_{T+1} options. Alternative approaches to the PPP variance estimation could be developed using the methods described in Rosenberg et al. (1992).

Calculating the Short-term Risk Function

One simple numerical scheme for combining the above results is as follows. First, define a series of catch "bins", each representing a narrow range of possible choices for this year's total allowable catch (TAC). Set up a cumulative probability array with an element for each catch bin and initialize the array to zero. Next, define a grid of S_T,M combinations that covers the full range considered physically possible, and for each combination on the grid, do the following: (1) run the iterated VPA, (2) compute the likelihood L^{qV} from eq. (11) and (12), and (3) for each value on a grid of B_{T+1} values, do the following: (3a) compute $p(B_{T+1}|S_T,M,D)$ from eq. (13)–(18), (3b) let $C^* = B_{T+1} - GS_T$, and (3c) add $L^{qV} p(B_{T+1}|S_T,M,D)$ to the cumulative probability bin representing C^*. After completing these steps for all S_T,M, add up the elements of the cumulative probability array into a total probability index T. Then, replace each array element with T minus the sum of all lower (smaller catch) array elements, divided by T. The array will then contain the (approximate) probabilities needed to plot

TABLE 1. Catch-at-age data for yellow perch in southern Green Bay, Lake Michigan. Catches in millions of fish.

Age	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
1	7.862	0.447	1.028	0.888	0.591	0.466	0.377	0.385	0.04144	0.0037	0.00276	0.0034	0.00896	0.0044	0
2	0.347	6.126	0.441	0.885	2.828	0.709	1.156	0.387	0.36424	0.92761	0.42202	0.30407	0.47512	0.52423	0.20377
3	0.533	0.045	1.144	0.221	0.584	0.327	0.334	1.884	0.23334	1.05082	1.02602	2.30035	1.31292	1.13957	0.84846
4	0.011	0.106	0.006	0.089	0.021	0.033	0.096	0.109	2.11101	0.15232	0.63247	0.58357	2.81831	1.64377	1.06755
5	0	0	0	0	0	0	0	0	0.03229	1.11417	0.18087	0.18567	0.3367	1.38579	0.35044
6	0	0	0	0	0	0	0	0	0.01162	0.0262	0.41022	0.03279	0.07216	0.19603	0.31028
7	0	0	0	0	0	0	0	0	0.00121	0.00049	0.01964	0.03674	0.01962	0.02814	0.03837
8	0	0	0	0	0	0	0	0	0.00005	0.00051	0.00098	0.00016	0.02281	0.00777	0.00292
9	0	0	0	0	0	0	0	0	0.00003	0.00007	0.00015	0.00008	0.00468	0.00096	0.00086

TABLE 2. Trawl survey estimates of relative abundance at age (thousands of fish/trawl hour) for yellow perch in southern Green Bay, Lake Michigan.

Age	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
0	0.639	0.862	1.112	0.197	5.737	0.196	0.409	4.519	11.302	0.836	2.331	0.626	0.983	2.749	0.48
1	1.512	0.168	0.677	0.493	0.231	2.117	0.344	0.764	2.603	1.667	0.942	0.717	0.372	0.39	1.055
2	0.021	0.521	0.095	0.115	0.238	0.205	0.871	0.135	0.406	0.375	1.376	0.16	0.204	0.229	0.296
3	0.001	0.003	0.058	0.009	0.039	0.044	0.02	0.57	0.044	0.037	0.22	0.297	0.071	0.098	0.106
4	0.0001	0.0001	0.0001	0.0004	0.0007	0.006	0.002	0.062	0.182	0.012	0.025	0.01	0.021	0.013	0.022

Fig. 1 (for each C bin, plot the array element against the catch corresponding to the lower limit or midpoint of the bin). Note that there should be a catch bin corresponding to negative or zero C^*; the cumulative probability associated with this bin is the probability that the stock will fail to increase (or decrease) by the factor G even if no catch is taken in year $T + 1$.

Example: Yellow Perch in Green Bay, Lake Michigan

The annual catch of yellow perch in southern Green Bay, Wisconsin (Table 1), is split roughly in half between a commercial net fishery that operates mainly in summer and selectively takes older fish and a recreational fishery that takes ages 1+ in summer and in a developing winter ice fishery. Management involves determining an annual allowable catch and allocating this between the gears; the commercial catch has been strictly monitored to avoid catches above allocation, while the sport fishery has been regulated through restrictions on daily bag and hooks per line. The fishery is generally viewed by users as being in a very healthy state following recovery from historical overfishing and pollution problems, and at least the commercial fishermen have been quite supportive of risk-averse policies to prevent a repetition of historical abuses. Thus, there has been demand for quantitative assessment of the risks associated with various TAC options; one approach to this assessment has been to use simulations based on earlier population estimates (before 1986) obtained from a regression method using the survey and catch data (Johnson et al. 1992).

Annual trawl surveys (Table 2) are conducted in late summer, after about two thirds of the annual catch has been taken ($N'_{at} = N_{at} - 0.67c_{at}$). The trawls take good numbers of age 0 fish, and hence constitute a prerecruit survey. Around 2000 fish are aged from the survey catch each year. Preliminary survey work indicated that few perch occupied deep waters (>20 m) during the 1970s, so the surveys were initially done mainly in shallower waters representing about 40% of the total habitat. Strong year classes produced during the 1980s were apparently associated with substantial dispersal of fish, especially older ages, into deep water; fishing pressure has followed this expansion, and the survey was extended in 1988 to include deepwater stations. Comparing the age compositions in Tables 1 and 2, it is obvious (and not surprising) that the inshore survey trawls have been selective for younger ages in general; very few age 5 and older fish appear in the surveys, yet fish up to age 9 are now common in the catch (around 5000–6000 fish are aged each year from catch sampling).

Methods of the previous section were applied using the data in Tables 1 and 2, along with the following conditions. The S_T,M grid was set up to represent uniform prior probabilities for S_T ranging from 0.1 times the 1992 catch to 10 times that catch (u_T from 0.1 to 0.9) and M ranging from 0.4 to 0.9 (s = 0.41–0.67; Johnson et al. (1992) estimated M = 0.56 using the Paloheimo (1980) method). For the iterated VPA, it was assumed that ages 6 and older have been equally vulnerable ($v_a = 1$) since 1988 and that the vulnerabilities of younger fish have not changed substantially since then (v_a terminal estimates obtained by averaging over 1988–1991 v_{at} estimates). The terminal number $N_{10,t}$ for

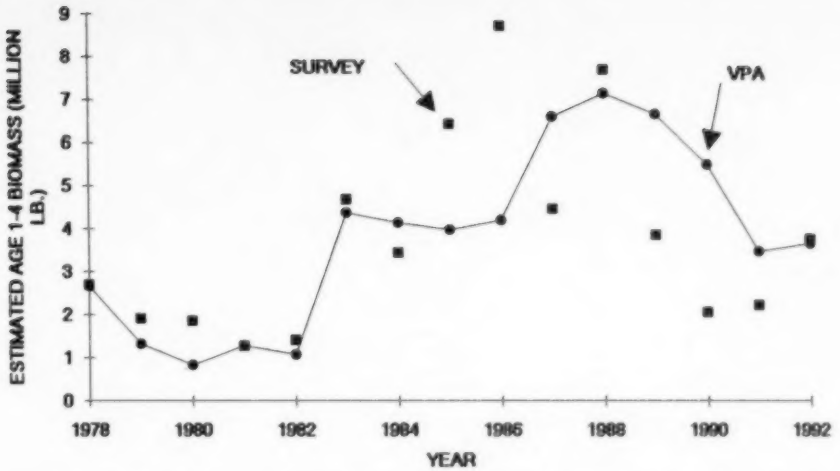

FIG. 2. Age 1–4 fall biomass of yellow perch in southern Green Bay. Values calculated from VPA numbers at age and survey catchabilities computed at the maximum likelihood combination of 1992 postharvest biomass (S_T) and natural mortality rate (M).

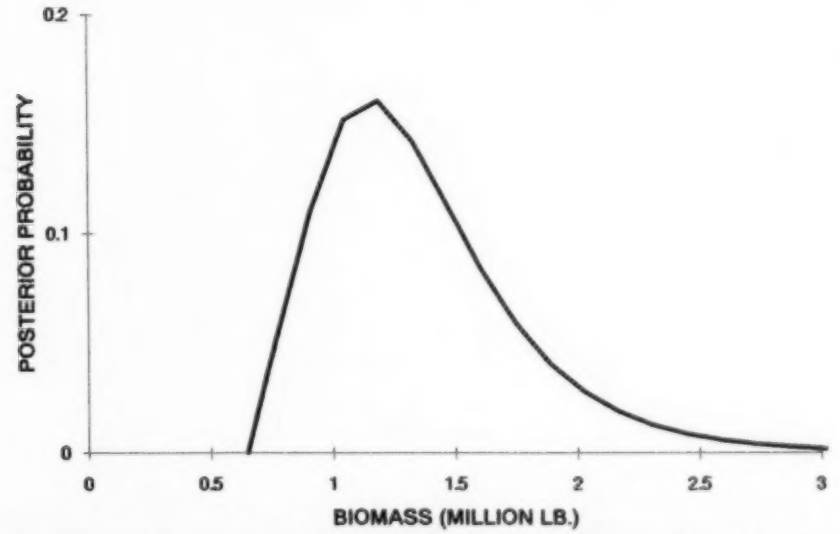

FIG. 3. Bayes posterior probability distribution for the 1992 preharvest biomass of yellow perch in Green Bay. Values are integrals over M of the posterior distribution $p(S_T,M|D)$ calculated as described in the text.

each cohort at age 10 was taken to be zero. For the weighted N_{aT} calculation (eq. (3)–(5)), the age composition sample size was assumed to be 5000 fish, and g_a was set to 1.0 for ages 5 and older. Only survey data for ages 1–4 were included in the likelihood calculation, and eq. (14) and (15) were used to predict $N_{1,T+1}$ (1993 age 1 recruits) from the age 0 survey data. PPP variance estimates (eq. (18)) were initially obtained for years $t = 1$ to $T - 1$, but the deviations for years before 1985 were discarded from the estimation when it was noticed that these were generally large and negative (u_t apparently much higher than u_T for the earlier t).

The computed risk function for $G = 1$ (odds of having same postharvest stock size in 1993 as in 1992) under the above conditions (Fig. 1) indicates fairly high odds of not being able to sustain even the relatively low 1992 catch (705 000 lb, 319 785.9 kg) in 1993. A tighter distribution was expected considering the extensive data available (Tables 1 and 2), and how well the age 1–4 biomasses computed from VPA agreed with those computed from the survey data and q estimates, evaluated at the maximum likelihood ("tuned") S_T,M combination (Fig. 2). In part, this is due to high uncertainty about S_T, as indicated by the marginal posterior distribution for $B_T = S_T + C_T$ integrated over M (Fig. 3); the broadness of this distribution indicates that the survey data can be almost equally well explained by (are almost equally likely given) a wide range of S_T "hypotheses". But a second and more important cause of uncertainty is that the conditional distribution of B_{T+1} given S_T,M is also

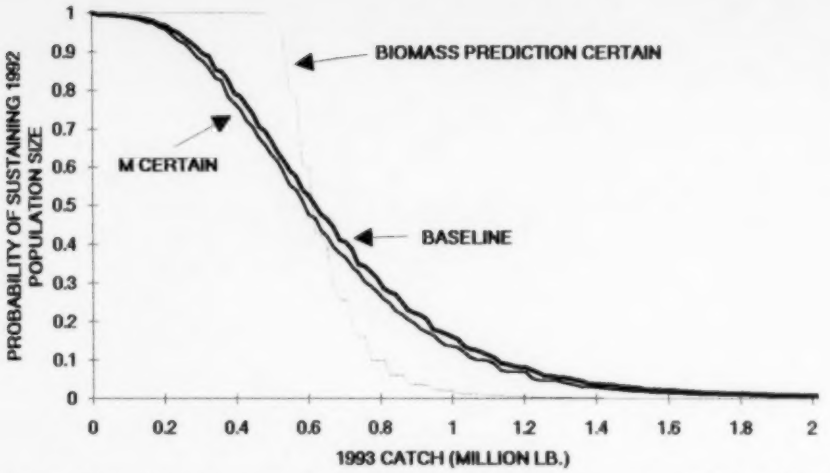

FIG. 4. Alternative assessments of the risk function for sustaining various 1993 catches of yellow perch in Green Bay. Light line next to the nominal (Fig. 1) estimate computed by ignoring uncertainty in M (with M fixed at 0.56). Dotted line computed by ignoring uncertainty in predicting 1993 preharvest biomass from 1992 postharvest biomass and recruitment.

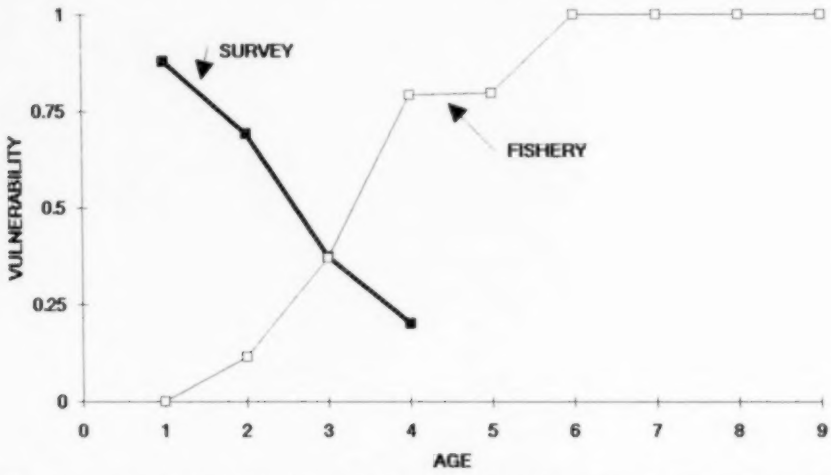

FIG. 5. Apparent relative vulnerability of yellow perch in southern Green Bay to the fishery and to trawl survey gear. Fishery estimates are iterated VPA estimates of vulnerability (v_a), and survey estimates are 10 times the conditional maximum likelihood estimates of survey catchabilities q_a; v_a and q_a were evaluated at the maximum likelihood combination S_T,M.

quite broad, at least as evidenced by the PPP variance; the calculated PPP variance implies a coefficient of variation of 10–20% in the B_{T+1} predictions, and ignoring this variation gives a substantially tighter risk function (Fig. 4). On the other hand, ignoring variation in M (assuming $M = 0.56$) does not lead to a much tigher risk function, when the PPP variance is included (Fig. 4).

Uncertainty in the B_{T+1} prediction given S_T,M is not due in this example to uncertainty about new recruits ($N_{1,T+1}$). The estimated relative vulnerability of age 1 animals is quite low (Fig. 5), so their contribution to B_{T+1} is insignificant. Also, d_{0t} is a good predictor of the $N_{1,t+1}$ recruitments estimated from VPA (Fig. 6), although there is some indication that survival rate of age 0 animals to recruitment at age 1 may be density dependent rather than constant as assumed in the eq. (14) and (15) prediction. The high PPP variance is due instead to the smorgasbord of errors mentioned above: c_{at} measurement errors, changes in v_a, time-varying s, and W_a, etc.

The w'_{at} and w_t deviations of the likelihood calculation suggest some violations of the independence assumptions made in calculating L^{qV} (Fig. 7). There are autocorrelated time effects w_t, and the w'_{at} deviations corrected for these time effects indicate some within-cohort correlations (most likely due to c_{at} sampling error effects on the VPA calculation of N'_{at}) as well as temporal patterns suggestive of cyclic changes in the survey catchabilities q_a. However, these correlation patterns are not necessarily a matter for serious concern; such patterns also appeared in the computed w'_{at} when the algorithm was tested (and found to work very

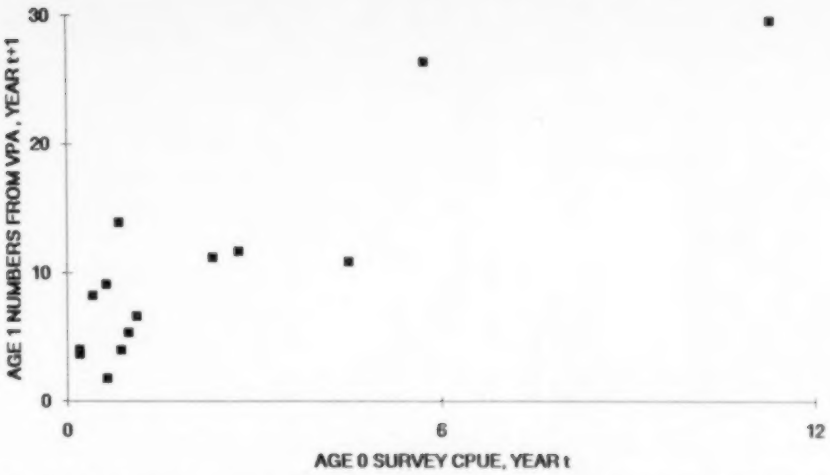

FIG. 6. Relationship between survey estimates of fall abundance of age 0 yellow perch in southern Green Bay and subsequent abundance of age 1 recruits estimated from VPA. VPA estimates shown are for maximum likelihood combination S_T,M; a similar pattern is evident for all S_T,M combinations considered in the analysis.

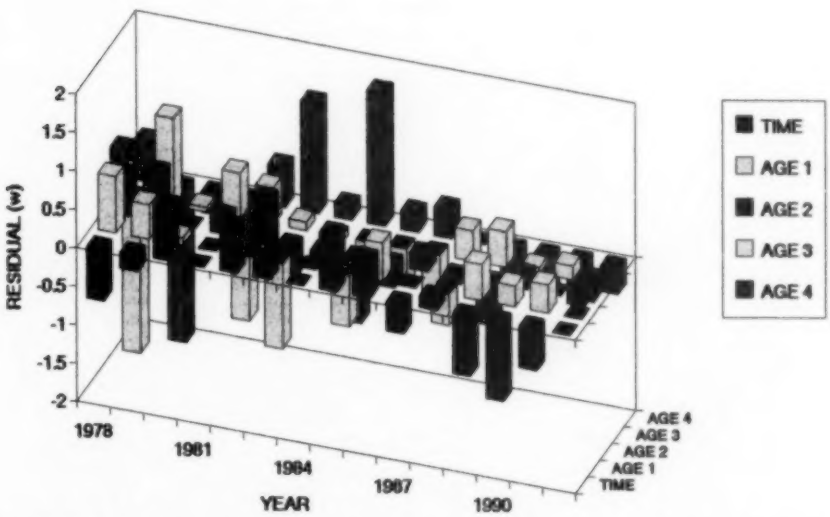

FIG. 7. Residual differences between log survey abundances and log of absolute abundances calculated by VPA, corrected for survey catchabilities, for yellow perch in Green Bay. First (front) residual series is shared time effect, w_t; series for ages 1–4 represent residuals w'_{at} corrected for shared time effect. Estimates shown are for maximum likelihood combination S_T,M.

well) with fake data generated without ageing errors and independent w_{at}.

Monte Carlo simulations of catch and survey sampling, from a population model with survival rates and harvest trends similar to those evident in the perch data, were conducted to provide estimates of the relative contributions of various error sources. These simulations indicated that roughly one third of the overall uncertainty in Fig. 1 is due to effects of multinomial sampling for c_{at} (assuming about 5000 fish have been aged per year). These sampling errors influence both the L^{qV} and PPP variance calculations, and hence propagate through the analysis to produce effects that are certainly substantial. However, the bulk of the uncertainty is due to a combination of survey errors (trawl and age sampling variation combined) and violations of VPA structural assumptions (changes in recent v_a,M).

The q_a estimates (Fig. 5) indicate that either vulnerability to the survey trawl decreases rapidly as fish grow or most fish move out of the shallow areas where the survey has been concentrated. Johnson et al. (1992) did not find such strong decreases in q_a with age in their analysis of the earlier data (before 1987), consistent with the idea that movement offshore of older fish has increased substantially with increases in stock size during the late 1980s.

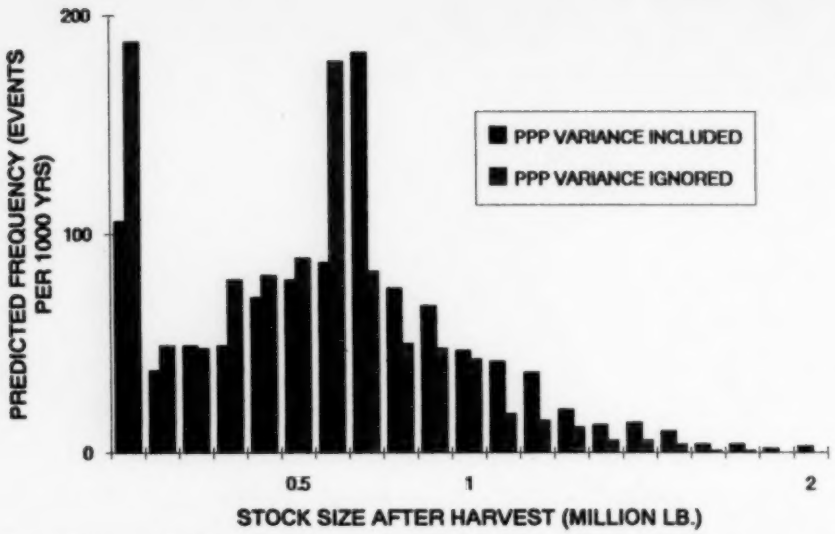

FIG. 8. Frequency distributions of postharvest stock sizes achieved by simulated future managers of yellow perch in Green Bay. Simulated manager in "include PPP variance" case uses full risk assessment calculation each year and achieves close to the target stock size about 70% of the time when aiming for 75% success. Simulated manager in the other case ignores variance indicated by past prediction performance and achieves a target less than 60% of the time.

Further Monte Carlo trials were conducted to insure that the risk function in Fig. 1 does in fact provide an accurate assessment of the probability of achieving S_T goals. Each trial consisted of 100 10-yr simulations of future perch management (1000 yr/trial). For each 10-yr simulation, we initialized a simulated population age structure at the 1992 maximum likelihood estimates. Then for each simulated year, we repeated the entire risk function analysis above and chose a quota for that year that should allow at least a 75% chance of achieving a postharvest target biomass of 0.5 million lb (0.2272727 million kg). This simulated a time-varying G strategy, aimed at stabilizing the stock size. We then generated surviving age structure and stochastic recruitment (lognormally distributed around historical average estimated from maximum likelihood N_{1t}) for the next simulation year, along with a multinomial catch age composition sample and a lognormally distributed survey abundance sample. Figure 8 shows typical distributions of "true" postharvest stock sizes from such trials. When the simulated manager uses the full risk function calculation, he achieves at least the 0.5 million lb goal in about 70% of years, which is remarkably close to the Fig. 1 prediction considering carryover effects that can occur after bad management errors (one very low stock size resulting from a quota overestimate can make it impossible to reach the target for at least 1 additional yr, even ignoring recruitment overfishing effects of such events). When the goal is raised to 0.7 million lb (0.319 million kg) or higher, the simulated manager achieves the goal in 80–85% of years, indicating the conservative effect of the PPP variance calculation when the stock is generally kept large enough to avoid carryover effects. In contrast, Fig. 8 also shows performance for a simulated manager who ignores the PPP variance calculation, in effect making just one deterministic prediction of B_{T+1} for each S_T possibility. This simulated manager ends up meeting the stock size objective in less than 60% of years, and in particular suffers a much higher risk of having very low stock sizes after harvest. Thus, while it may be discouraging to see the very flat risk function (high risk over wide range of catch) resulting from the full analysis, it is perilous for long-term management to pretend greater certainty than the function indicates.

To this point, the analysis appears to produce a consistent, if somewhat pessimistic, picture of uncertainty about the fishery. But a very serious uncertainty about how to do the risk calculation is shown in Fig. 9. This figure shows alternative risk functions calculated by replacing the N_{aT} calculation of eq. (3) with the extreme alternatives represented by $g_a = 1$ (all weight on the iterated or separable VPA estimator) versus $g_a = 0$ (all weight for younger ages placed on the age composition indicated by survey data). These alternatives should both be close to the result in Fig. 1 if the assumptions about constant survey catchabilities q_a are indeed valid. But the survey data taken alone indicate a relatively low, precise estimate of S_T (and very high u_T), while the c_{at} data alone are consistent with a higher, much more uncertain estimate. This inconsistency is not due just to the 1992 c_{aT} or d_{aT} data; a similar pattern is obtained for T = 1990 or 1991. Similar inconsistencies, and patterns in w_t like Fig. 7 (negative transient of w_t during late 1980s), are easily produced in fake test data just by reducing all the q_a values for the last several simulated years. The apparent movement of fish into deeper water mentioned above would cause such a decrease in the q_a for inshore survey sites, as would incorrect weighting of inshore versus offshore data in constructing the average survey index d_{at}. Further support for this interpretation is provided by the c_{at} data; the continued presence of older fish in the catch is not consistent with the high u_t values deemed most likely when $g_a = 0$ (but such patterns could also be explained by increasing vulnerability with age).

As in a number of fisheries where survey data have been

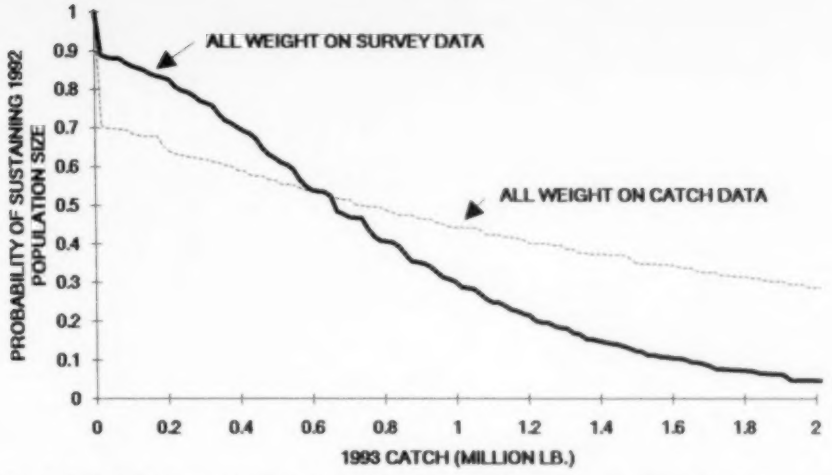

FIG. 9. Pathological sensitivity of the risk function calculation to method used to estimate terminal age composition for VPA for yellow perch in Green Bay. Function that looks much like Fig. 1 calculated by initializing VPA ages 1–4 from 1992 survey data only; much flatter function calculated by initializing VPA with 1992 age 1–4 catch data only. Discrepancy likely due to changes in survey catchabilities in recent years; result similar to flatter function is obtained by discarding survey data for years prior to 1987.

used in conjunction with VPA, we are left in this example with the nasty question of whether or not to trust the basic survey design and the assumption that the survey indices are actually proportional to abundance. Are the declines in abundance indicated by the survey "real", or have the fish simply moved into a "black hole" not adequately covered by the survey? One conservative option to represent this uncertainty is to simply discard the older survey data, which results in a much broader posterior distribution for S_T,M and a correspondingly flatter risk function. This option will certainly prevent any overly optimistic assesment of risk. Another option is to replace d_{at} with a "judgemental" survey time series constructed with best judgemental estimates (guesses) for the survey catch rates that would have been obtained in those strata/areas/years that were not covered in the original survey design, using auxiliary observations or expert judgement about when fish started using (or disappeared from) those areas. Obviously this option could produce overly optimistic risk assessments, but does make use of various sources of information that are typically available in addition to the survey data. Such a judgemental time series has been constructed for Green Bay perch (B. Belonger, Wisconsin DNR, Green Bay, and C. Kraft, University of Wisconsin, Green Bay, personal communication), and using it does indeed appear to resolve some of the anomalies mentioned above (survey q's appear flatter with age; higher terminal stock size and lower exploitation rate appear probable for all assumptions used to estimate N_{aT}). However, we hesitate to present such results in further detail, since they could be very misleading; more work needs to be done on possible biases and risks associated with using such judgemental series.

Conclusion

The methods developed above can be used to provide conservative assessments of the short-term risk associated with various TAC choices. But like other assessment procedures that use relative abundance information in conjunction with catches (rather than direct measurements of absolute abundance), the results are critically dependent on having relative abundance indices that truly are proportional on average to absolute abundance. There is no use hoping that more elaborate statistical methodologies or VPA tuning procedures will somehow substitute for good survey design. Further, the key survey design problem is not how to increase survey precision by taking more observations in strata/areas where fish are known to occur, but rather how to make sure that the survey covers a broad enough range of areas to detect major shifts in fish distribution. This means either "wasting" a great deal of time gathering negative (no fish) observations in areas not currently occupied or developing multistage, adaptive survey designs that use some quick ways to test for changes in distribution followed by more detailed sampling based on such tests. At present, the development of such survey designs is a far more important challenge for fisheries scientists than the development of better models for risk assessment.

It is possible of course to avoid using survey data in the first place, by setting the VPA terminal exploitation rates under some regularity assumption or by using fishery catch-per-unit-effort (CPUE) data as the relative abundance index. But regularity assumptions (such as constant fishing rate over last few years to provide a separable VPA structure, or assuming terminal fishing rate proportional to some effort measure) are extremely risky for most modern fisheries, where efficient fishing methods imply that exploitation rates can increase rapidly during periods of stock decline. The same objection applies to using CPUE data; increasing catchability over time or with declining stock size can easily produce grossly optimistic estimates of the current stock size.

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Appendix. Approximation of Likelihood Function Without Integration over Process Nuisance Parameters

The likelihood function L^{qV} is an approximation in that uncertainty about the "nuisance" parameters $N_{a,T}$ given S_T (the age composition of S_T) is ignored through substitution of the maximum likelihood estimators (eq. (3)) for them into the likelihood calculation, instead of integrating over them or treating them as leading parameters (like S_T) in the overall analysis. It is not numerically practical to treat them as leading parameters, so some integration or approximation method is necessary. Using the maximum likelihood estimators is motivated by the following argument.

Suppose that the model parameters θ are divided into two subsets θ_1 and θ_2, where θ_1 consists of leading parameters such as S_T and M, while θ_2 consists of nuisance parameters such as age proportions of S_T. Ideally, posterior distribution calculations and inferences related to θ_1 should be based on the marginal likelihood "kernel" L^{θ_2} obtained by integrating over the nuisance parameters (here the prior probability density p_0 would ordinarily be assumed to be flat):

$$(A1) \quad L^{\theta_2} = \int_{\theta_2} L(D|\theta_1,\theta_2)p_0(\theta_2)\, d\theta_2.$$

Integrations of this sort present major numerical problems when the set θ_2 contains many parameters and cannot be done analytically for parameters influencing the dynamic predictions N_{at} to provide simple formulae like Walters and Ludwig (1994) provided for the observation parameters q,V. However, the integral in eq. (A1) can be represented as two components:

$$(A2) \quad L^{\theta_2} = L(D|\theta_1,\theta_2)H(D,\theta_1)$$

where the first component is the approximating likelihood function for D given θ_1 obtained by substituting the maximum likelihood estimators for θ_2 at each point where these parameters appear, and $H(D,\theta_1)$ is a constant of integration that may depend on θ_1. H measures the spread or variance of the posterior distribution for θ with respect to θ_2 at each θ_1 point in the parameter space. If H is independent of θ_1 (as occurs for the q,V observation parameters in Walters and Ludwig 1994), then it can be ignored and the posterior distribution for θ_1 is exactly that obtained by substituting the maximum likelihood estimators of θ_2 into the overall likelihood function for D given θ.

So if there is concern about the approximation, a simple test strategy is to carry out the tedious numerical integration over θ_2 for a few values of θ_1 that are assigned nontrivial probabilities by the approximate calculation and look to see if H changes much over these values. For the yellow perch data, this test strategy indicates that H is quite flat (independent of θ_1) for most $S_{T,M}$ combinations, but does drop off for very low S_T and high M values. Such decreases in H at extreme parameter combinations imply that the approximate likelihood calculation is actually conservative, assigning higher posterior probabilities to more extreme parameter combinations than would be obtained using a complete integration method; however, this is not a general result. Usually, integration over one subset of parameters will produce a posterior distribution for the other subset that is broader than predicted by the maximum likelihood approximation.

Infection with the Piroplasm *Haemohormidium terranovae* in Relation to Haematocrit and Mortality of American Plaice (*Hippoglossoides platessoides*)

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The relationship between infection with *Haemohormidium terranovae* and the health of captive American plaice (*Hippoglossoides platessoides*) was investigated for over a 1-yr period. Intensity of infection and haematocrit were not significantly correlated at any of the sampling periods, although there was a moderately negative relationship between these parameters when data from all periods were pooled. Intensity and haematocrit varied inversely in time; intensities rose gradually during the winter months and dropped off significantly in the summer, while haematocrit values fell significantly until March and increased significantly by June. Only haematocrit showed a significant correlation with water temperature. Fish harboured higher intensities and lower haematocrit values prior to death than was apparent in fish that survived. The greatest differences between the mortality-group and the survivor-group occurred just prior to the greatest number of deaths, between March and June. The presence of infection in all captive plaice at all sampling periods, in the absence of leeches, is discussed in relation to possible mechanisms for long-term maintenance of infection and to possible routes of transmission of this parasite.

Le rapport entre l'infection par *Haemohormidium terranovae* et l'état de santé de plies d'Amérique (*Hippoglossoides platessoides*) captives a été étudié sur une période de plus d'un an. En aucune période d'échantillonnage existe-t-il une corrélation significative entre le degré d'infection et l'hématocrite même s'il existe un rapport inverse modéré entre ces paramètres lorsque les données de toutes les périodes sont regroupées. Le degré d'infection et l'hématocrite varient à l'inverse l'un de l'autre en fonction du temps; le degré d'infection s'accroît graduellement au cours des mois d'hiver et chute à l'été tandis que l'hématocrite diminue considérablement jusqu'en mars et s'est accru considérablement en juin. Les poissons qui étaient sur le point de mourir étaient plus infestés et avaient des hématocrites moins élevés que ceux des poissons qui ont survécu. Les plus grands écarts entre le groupe des survivants et celui de ceux qui sont morts ont été observés juste avant la période du plus grand nombre de décès, soit entre mars et juin. L'existence de l'infection dans toutes les plies captives à toutes les périodes d'échantillonnage, en l'absence des sangsues, est examinée en fonction de possibles mécanismes de persistance à long terme de l'infection et de vecteurs possibles de transmission de ce parasite.

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Henry (1910) first described *Haemohormidium cotti* and *Haematractidium scombri* from sculpins (*Cottus* spp.) and Atlantic mackerel (*Scomber scombrus*), respectively, from the Irish Sea. These parasites were subsequently grouped with piroplasms in the protistan phylum Apicomplexa. Certain piroplasms are known to be pathogens. *Babesia bigemina*, for example, is responsible for "red water fever" in cattle in Texas, South America, and Australia (Mehlhorn and Schein 1984). Little is known of the host–parasite interactions of fish piroplasms. Their ultrastructural features have been described (Johnston 1975; Davies 1980; Bodammer and MacLean 1985) and there are reports of successful biological transmission facilitated by blood-feeding piscicolid leeches (Khan 1980, 1984). With the exception of Khan's (1980) 6-mo study of captive eelpout (*Lycodes lavalaei*) harbouring natural infections of *Haemohormidium beckeri*, there are no data indicating temporal patterns of parasitaemia of these protists; neither are there data relating infection to the health status of fish hosts.

American plaice (*Hippoglossoides platessoides*) are right-eyed pleuronectiforms found along northwestern Atlantic coastal areas (Scott and Scott 1988) and are under consideration for coastal aquaculture purposes (e.g., Audet et al. 1993). This species harbours natural infections of *Haemo-*

hormidium terranovae along much of the Canadian coast from Davis Strait to George's Bank (So 1972; Khan 1980, 1984; Khan et al. 1980, 1991). Because disease produced by the piroplasms of mammals involves anaemia as a result of rapid destruction of red blood cells (Babès 1888; Wilde 1967), we followed *H. terranovae* infections in plaice in relation to both haematocrit levels and mortality of these fish in captivity.

Materials and Methods

Sample Collection

Fish were captured on June 13, 1991, in the St. Lawrence River estuary, 3–4 km offshore of Matane, Quebec (48°50′N, 67°33′W), by Yankee bottom-trawl at a depth of 45 m. About 400 American plaice were transported to the Maurice-Lamontagne Institut (IML) near Mont-Joli, Quebec, and maintained in captivity in five separate 550-L flow-through holding tanks. The saltwater intake for IML is situated 2 km offshore and at a depth of 15 m. Water is filtered five times using a sand filtration system (particle size ~1 mm). A total of 100 plaice (20 from each of five holding tanks) were chosen randomly and marked with a numbered tag using a Dennison™ tagging apparatus. Total length (snout to tip of tail) was recorded for each fish. Plaice were fed defrosted capelin (*Mallotus villosus*) or shrimp (*Pandalus borealis*) once or twice per week and were monitored daily for morbidities and mortalities.

Data obtained prior to August 1991 were not considered in order to eliminate confounding effects of acclimation to the captive environment. On six dates, separated by approximately 2 mo each (August 26, 1991; October 31, 1991; January 9, 1992; March 13, 1992; June 12, 1992; and September 10, 1992), less than 0.2 mL of blood was taken from each surviving tagged fish. Due to accidental death of the tagged study group, data for those fish could not be followed beyond October 16, 1992. In December 1992 and in March 1993, however, 20 fish were sampled randomly from untagged fish which also had been held in captivity from June 1991. Blood was withdrawn from the caudal vein or artery using a heparinized syringe and a 25-gauge needle inserted on the right side midbody below the vertebral column. Blood was dispensed into heparinized capillary tubes and thin blood films were made on glass slides. Capillary tubes were sealed on one end with Crit-O-Seal™ and centrifuged at 6000 rpm for 3 min to determine haematocrit (Biofuge™, Baxter Scientific Products). Intensity of infection with *H. terranovae* was determined from blood films stained with Diff-Quik™ (Baxter Scientific Products) by determining the proportion of infected erythrocytes and erythroblasts in each blood film with the oil immersion objective of a compound microscope (magnification = 1250×) using an ocular grid. Approximately 500 cells were examined across two separate transects of each blood film made. In the event that parasites were not observed in the 1000 erythrocytes, the slide was examined for up to an additional 30 min. Poor staining prevented accurate intensity counts for August 1991.

Analyses

Statistical tests were performed with Systat™ and Number Cruncher Statistical System (Hintze 1986) software, unless otherwise specified. The relationship between haematocrit and intensity of infection for individual fish within each sampling interval was examined from scatterplots and analysis of distance-weighted least-squares (DWLS) to look for trends. Data of intensities of infection demonstrated skewness (G1) and leptokurtosis (G2) and were normalized with a square-root transformation. The justification of this transformation (Sokal and Rohlf 1981) is twofold: raw data are counts, and their means and variances showed significant covariance. Although haematocrit data were approximately normal, because they are binomial, and thus variances are functions of means, they were adjusted through the arcsine transformation. Repeated-measures analysis of variance (ANOVA) was used as opposed to standard ANOVA because the former design is appropriate where individuals in each treatment group are the same whereas the latter assumes independence of the individuals in each treatment group. For all parametric repeated-measures ANOVA, a priori tests for homoscedasticity (homogeneity of variance) were carried out for each measure repeated by comparison of the maximum variance ratio (MVR) and appropriate critical values of the F_{max} statistic (Hartley 1950; Sokal and Rohlf 1981). To determine if there was a significant change over time in the population intensity of *H. terranovae*, or in the population haematocrit levels, repeated measures ANOVAs were employed for each, using the five holding tanks in which they resided as a blocking term. In addition, the effect of total length was examined as a covariate in these analyses. A posteriori tests for multiple comparisons (Newman–Keul's and Fisher's LSD) were used to locate which of the pairwise differences were statistically significant. The overall relationship between haematocrit and intensity was assessed with Pearson's product-moment correlation statistic. The relationships between ambient water temperature and either haematocrit or intensity were assessed by simple first-order regression.

Repeated-measures ANOVAs were also used to assess whether fish that died over the course of the study (mortality-group) differed from those that did not (survivor-group) with respect to intensity or haematocrit. The mortality-group included, for each period, any surviving fish which was known, a posteriori, to have eventually died. Four fish were excluded from the mortality-group and from the survivor-group (two were inadvertently killed, one died during a methylene-blue treatment for fungal infections, and one vaulted from its holding tank). Finally, the relationship between initial size and mortality was assessed with a *t*-test comparing the mean total lengths of the mortality-group and the survivor-group.

Results

Haematocrit levels observed during study periods ranged from 1.2 to 24.5%. Intensity of infection with *H. terranovae* ranged from less than 0.01% of erythrocytes infected to 13.3% infected. All tagged fish had infections when captured and all surviving fish remained infected throughout the study periods. All randomly sampled fish examined in December 1992 and in March 1993 also were infected.

There were no consistent patterns in the relationship between parasitaemia and haematocrit for individual fish at each sampling period. Curves fit to the data did not suggest consistent patterns.

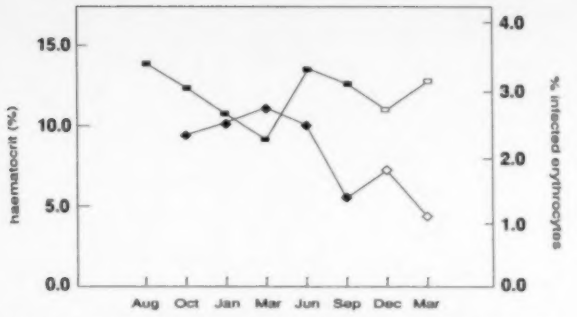

FIG. 1. General trends observed in median haematocrit (squares) and intensity (diamonds) across sampling periods. The study group is represented by values obtained prior to October 1992 (solid symbols). Data shown for December 1992 and March 1993 (open symbols) represent 20 fish selected randomly from fish captured in June 1991.

Population Intensity and Haematocrit

Trends observed in the values of both intensity and haematocrit suggested that these parameters varied inversely in the population of tagged fish over the sampling periods as well as for the two randomly selected groups that followed (Fig. 1). After square-root transformation of intensity and arcsine transformation of haematocrit, a priori tests for homoscedasticity yielded MVRs of 2.45 for haematocrit and 2.00 for intensity across sampling periods. Both of these fell short of the critival values of F_{max} (where $\alpha = 0.05$) needed to reject the hypothesis that variances were not different.

Product-moment correlation coefficients for haematocrit and intensity (transformed data) within sampling periods, although all negative, were not significant ($0.188 < p < 0.584$), while data pooled for all study periods showed a moderately negative relationship between the two parameters ($r = -0.116$, $p = 0.077$). The relationship between water temperature (Fig. 2A) and haematocrit was strongly positive ($r = 0.983$, $p = 0.003$), while that between water temperature and intensity, although negative ($r = -0.474$), was not significant.

Repeated-measures ANOVA, in which the five tanks were held as a blocking term, indicated that there were differences ($p < 0.001$) in haematocrit between sampling periods (Fig. 2B) and that the tank in which the fish were held had no effect ($p = 0.215$); neither did the total length of the fish ($p = 0.943$). Haematocrit values during August and October 1991 as well as June 1992 were higher (Neuman–Keul's and Fisher's LSD tests, $p < 0.05$) than those during January and March 1992. Equivalent tests used for intensity of infection with *H. terranovae* (Fig. 2C) revealed significant differences across sampling periods ($p = 0.008$) and no effect of tanks ($p = 0.119$) or of total length ($p = 0.652$) on intensity. A posteriori tests for multiple comparisons indicated that the only significant change in parasitaemia was a decline from June to September 1992. Interaction terms (sampling period × tanks) were not significant in either case.

Mortality

During the study interval, 26 fish died. Of those 26, four were attributed to unrelated causes and were excluded from

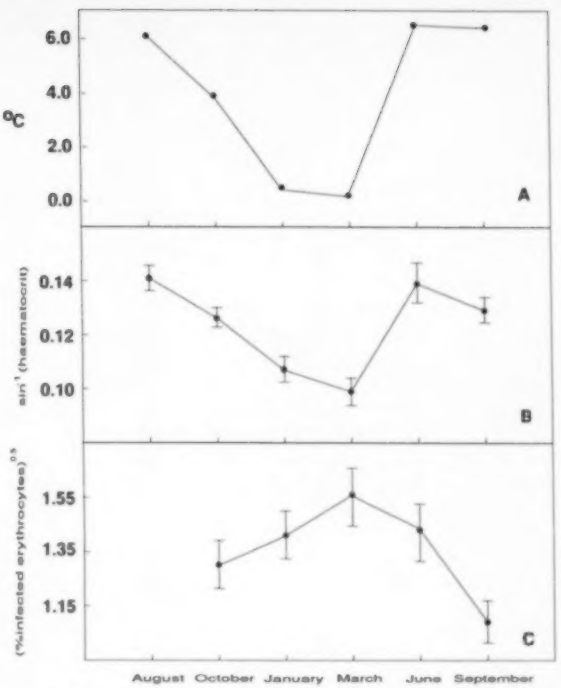

FIG. 2. Seasonal changes over the study period in (A) temperature, (B) mean arcsine transformed haematocrit, and (C) mean square-root transformed intensity. Bars = standard error.

analyses. All tagged fish died on October 16, 1992, due to a faulty drain valve. The greatest number of deaths (11) occurred in the interval between March and June 1992; two to four deaths occurred in each of the other intervals.

In the mortality-group, mean haematocrit levels were consistently lower (Fig. 3) and mean intensity of *H. terranovae* consistently higher (Fig. 4) than in the survivor-group. In a priori tests of homoscedasticity, MVRs of the mortality-group and the survivor-group were 5.10 and 1.81, respectively, for haematocrit and 1.54 and 1.33, respectively, for intensity, none of which were sufficiently large to reject the hypothesis that variances across sampling periods for these groups were not different. Repeated-measures ANOVA indicated that haematocrit levels in the mortality-group were significantly lower than those in the survivor-group ($p = 0.025$). Conversely, the intensity levels in the mortality-group were significantly higher than those of the survivor-group ($p = 0.005$). Interaction terms for the haematocrit and intensity analyses were negligible ($p = 0.786$ and 0.384, respectively). The greatest difference between these two groups, both for intensity and for haematocrit, occurred in the March 1992 sampling period (Fig. 3 and 4).

Fish in the mortality-group ($\mu = 27.9$ cm) were longer ($p = 0.030$) at the time of capture than those in the survivor-group ($\mu = 25.88$ cm).

Discussion

Our most unexpected finding was that all *H. platessoides* remained infected with *H. terranovae* throughout the study and that the intensity of some infections rose considerably

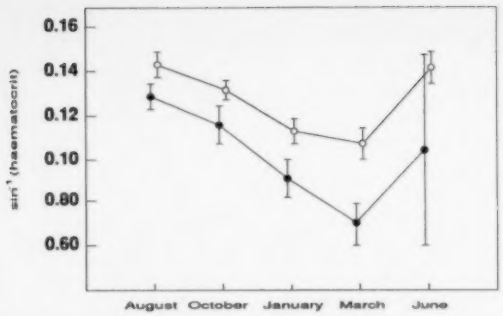

FIG. 3. Comparison of mean arcsine transformed haematocrit in the mortality-group (solid circles) and in the survivor-group (open circles). Bars = standard error.

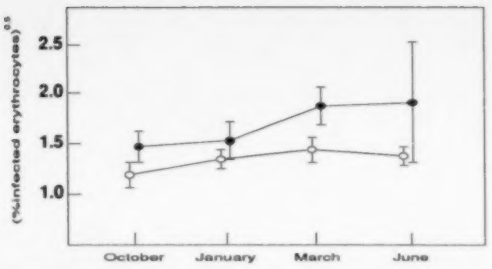

FIG. 4. Comparison of mean square-root transformed intensity in the mortality-group (solid circles) and in the survivor-group (open circles). Bars = standard error.

from one sampling period to the next. Moreover, data from randomly sampled fish in December 1992 and March 1993 support the view that American plaice are not able to eliminate infections even after 2 yr in captivity. This is surprising given the apparent role of blood-feeding leeches in completing the infection cycle (Khan 1980, 1984). All fish were free of leeches when they were captured. Furthermore, the filtration system at IML excludes leeches which may otherwise maintain or exacerbate infections. It is possible that once fed upon by a leech carrying *H. terranovae*, fish remain infected for the duration of their lives. This capacity for long-term maintenance of infection has been well documented in malarial parasites (Chernin 1952; Desser et al. 1967; Garnham 1967). Infections with *H. terranovae* did not, however, show the episodic remission and recrudescence that are characteristic of *Plasmodium* species. Immunity of the infected host may be maintained in a state of premunition, insufficient in strength to eliminate the parasites and yet capable of preventing sustained high intensities. The significant decrease of intensity over the summer months (Fig. 2C) supports this notion, as the immune system is more effective in warmer periods (Serero and Avtalion 1978). The inability of any fish to achieve sterile immunity, however, seems unlikely. Another hypothesis is that leeches are not involved, or are not necessary, in transmission of this parasite among fish. This is consistent with finding 100% prevalence in the absence of leeches at the time of capture. This would contradict Khan's (1984) report of successful transmission of *H. terranovae* from plaice to winter flounder (*Pleuronectes americanus*) by the leech *Johanssonia arctica* and of *H. beckeri* from eelpout to both longhorn sculpin (*Myoxocephalus octodecemspinosus* and Atlantic wolffish (*Anarhichas lupus*) through the leech (*Platybdella olriki* (see Khan 1980). On the other hand, whereas leeches were first implicated in the transmission of *Cryptobia salmositica*, it has become clear that transmission can also occur through water contamination in the absence of vectors (Bower and Margolis 1983; Woo and Wehnert 1983).

Seasonal Intensity and Haematocrit

The trends observed in the plaice population for intensity of infection with *H. terranovae* are similar to data obtained for *H. beckeri* infections (Khan 1980), with a generalized enhancement of intensity through the winter months and a significant drop between early and late summer. At least three of the six fish examined showed the same pattern (Khan 1980). Two eelpout in that study did not show infection during 1–3 mo before *H. beckeri* became patent, and half of the study group apparently eliminated their infections. Similarly, winter flounder infected with *H. terranovae* eliminate infections after 13–72 d (Khan 1984). Those data may need to be reinterpreted because 8 plaice in our study showed fewer than one infected cell in the 1000 cells observed during intensity counts. For these, the blood films had been examined thoroughly for parasites, and in all cases, parasites were eventually found. Only one fish remained at this low level; the rest later demonstrated intensities that were 2 orders of magnitude higher. One of these had harboured parasitaemias lower that 0.01% for 10 mo prior to showing an intensity of 2.7%. In light of these observations, the capture of plaice during warmer months for aquacultural purposes, designed to avoid infections with *H. terranovae*, would be misdirected, as undetectable infections would become evident later. Similarly, the examination of fish stocks for prevalence of these parasites (e.g., Khan 1980; Khan et al. 1980, 1991, 1992) needs to be interpreted with caution, as the determination of prevalence will be a function of the ease with which infections are detectable.

Seasonal changes observed in haematocrit values are similar to those reported by Audet et al. (1993); their results, however, did not indicate that the highest haematocrit values coincided with the highest water temperature. In contrast, for our study group, the highest and lowest haematocrit values corresponded to the periods of highest and lowest water temperatures, respectively (Fig. 2). This difference may be accounted for when differences in sampling periods are considered. Changes in haematocrit levels of Atlantic cod (*Gadus morhua*) have been explained as an adaptive response to changes in oxygen demand that ensues with changes in water temperature (Lie et al. 1989). Although this appears to be supported here, existing discrepancies have been highlighted (Audet et al. 1993). Moreover, no correlation was found between haematocrit and water temperature for Atlantic salmon (*Salmo salar*) (see Sandnes et al. 1988), and the opposite relationship has been observed for winter flounder (see Mahoney and McNulty 1992).

The relationship between haematocrit and intensity was complex. These parameters showed opposite trends with respect to fluctuations over the sampling period (Fig. 1 and 2) and in relation to deaths of the captive fish (Fig. 3 and 4), but when compared directly, this negative relationship was not strong enough to state unequivocally that haematocrit values were affected by infection with *H. terranovae*. Haematocrit is a secondary whole-animal response

to stress (Barton and Iwama 1991). Reports of significantly lower haematocrit in association with disease include winter flounder with fin necrosis (Mahoney and McNulty 1992) and ducks infected with intraerythrocytic parasites (Maley and Desser 1977). Although sea bass (*Dicentrarchus labrax*) show decreased haematocrit with a combination of viral, bacterial, and parasitic infections, they do not when infected with any single pathogen (Alverez-Pellitero and Pinto 1987). In addition to disease, low haematocrit values in fish are induced by a variety of stressors such as sudden alterations to salinity (Davis and Simco 1976), chemically or thermally induces anoxia with acute respiratory stress (Murad et al. 1990), and exposure to sewage effluent (Buckley et al. 1976). If intensity of infection with *H. terranovae* is, in fact, causing anaemia in plaice, the variety of potentially interfering variables would make it difficult to detect. Nevertheless, our observations may provide some insight into the questions raised by Audet et al. (1993) regarding the health status of captive plaice which stemmed from their observations of haematocrit levels and other blood parameters.

Mortality

The patterns observed both for intensities of *H. terranovae* and for haematocrit in relation to mortality are evident from a number of our analyses. In addition to the mortality-group harbouring higher intensities and being anaemic for a sustained period prior to death (Fig. 3 and 4), the observed differences between the two groups were greatest, both for intensity and for haematocrit, in the March 1992 sampling period which preceded the greatest number of deaths in the study group. These patterns are suggestive of a chronic condition in certain fish as opposed to mortality resulting from acute pathogenesis. Late spring appears to be a period when plaice are particularly vulnerable. This may be related to lack of nutritional reserves following the prolonged period of relatively low food intake through winter, or to generalized changes in physiology which correspond to reactivation of the immune system or to plasma sterol levels related to spawning in this period (Pitt 1966).

To conclude that *H. terranovae* is directly contributing to the deaths of captive plaice would be premature. This issue would have to be investigated through controlled studies involving uninfected, naturally infected, and experimentally infected plaice. Inasmuch as prevalence of this parasite was 100% in *H. platessoides*, this would be difficult to achieve without the use of fish raised in captivity. On the other hand, it is clear that factors contributing to mortality and anaemia are also playing a role in higher intensities of *H. terranovae*.

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Trends in the Abundance of Chinook Salmon (*Oncorhynchus tshawytscha*) of the Nechako River, British Columbia

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Trends in abundance of chinook salmon (*Oncorhynchus tshawytscha*) of the Nechako River, a tributary of the Fraser River, were analyzed to quantify the ecological effects of water abstraction for electricity generation. In years when the majority of returning chinook adults used the upper Nechako River for spawning, the survival of offspring for the entire river was poorer than in years when spawning was concentrated in the lower reaches. Relative to the historical discharge, the upper Nechako River has experienced the greatest degree of water abstraction, and the lower survival of chinook broods originating from the upper river may be due to early emergence of fry caused by elevated fall and winter water temperatures or to higher rates of predation on juveniles and loss of rearing habitat caused by the elimination of the spring freshet. Poor recruitment resulting from broods spawning predominately in the upper river has caused the trend in the abundance of Nechako River chinook to diverge from the trend of chinook populations of similar life history from unregulated tributaries of the Fraser River. Additional reductions in flow may further affect the capacity of the upper Nechako River to produce chinook salmon.

On a analysé les courbes d'abondance du saumon quinnat (*Oncorhynchus tshawytscha*) de la rivière Nechako, un tributaire de la rivière Fraser, afin de quantifier les effets écologiques de prélèvements d'eau pour la production d'électricité. Les années où la majorité des adultes quinnat est remontée jusque dans la partie amont de la rivière Nechako pour la ponte, la survie de la descendance dans l'ensemble du cours d'eau était inférieure à ce qu'elle était les années que la ponte était concentrée dans la partie aval. Comparativement au débit historique, le débit de la partie amont de la rivière Nechako est à son plus bas à cause du grand prélèvement d'eau, et le taux inférieur de survie de la progéniture qui provenait de ce secteur peut être attribuable à une émergence hâtive des alevins qui dépendrait de la température élevée de l'eau durant l'automne et l'hiver ou encore au taux élevé de la prédation qui s'est exercée sur les juvéniles, ainsi qu'à la perte de zones d'élevage par suite de l'élimination de la crue nivale. Le mauvais recrutement, attribuable à la situation de la progéniture qui provient principalement du secteur amont du cours d'eau, est à l'origine d'une divergence entre la courbe d'abondance du quinnat de la rivière Nechako et celles de populations qui ont un cycle biologique semblable, mais qui vivent dans des tributaires non régulés de la rivière Fraser. De nouvelles réductions du débit pourraient nuire davantage à la capacité du secteur amont de la rivière Nechako de produire du quinnat.

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The Nechako River, a tributary of the Fraser River that has a chinook salmon (*Oncorhynchus tshawytscha*) run of approximately 1900 spawners (1974–91 average), is currently under intensive study to monitor the effects of flow diversion for hydroelectric power generation. The existing diversion project is the largest in the Fraser River watershed, and considerable concern has been expressed about the potential effect of further flow modification on chinook and other salmonid populations that use the Nechako watershed for migration, spawning, and rearing (Mundie and Bell-Irving 1986).

The Nechako River was dammed in 1952 at a point 280 km upstream from the confluence with the Fraser River (Fig. 1). After the reservoir filled, about 40–50% of the Nechako flow was diverted to turbines on the Kemano River watershed for hydropower (Jaremovic and Rowland 1988). In 1978, flows were further reduced for additional electricity generation, and since 1980 the discharge has been dictated by legal requirements and agreements between the Department of Fisheries and Oceans (DFO) and the developer. During this recent period, flows have been relatively stable throughout most of the year, except for releases made in late July and early August to cool the lower portion of the river for sockeye salmon (*Oncorhynchus nerka*) migrating to the Stuart and Stellako Rivers, tributaries of the Nechako (Fig. 1 and 2). In 1987, an agreement was reached between the developer, DFO, and provincial authorities for another reduction in flow for an expansion of hydroelectric capacity. The proposed schedule of discharges will result in annual flows of 10–20% of the historical annual mean (Nechako Fisheries Conservation Program (NFCP) 1988).

Here I present an analysis of recent trends in abundance of the Nechako chinook stock and an interpretation of possible effects of flow on survival. The effects of flow diversion on this stock have been difficult to estimate because the main measure of abundance, the number of adult spawners, is affected by unknown variation in both marine survival and fishing mortality (Mundie and Bell-Irving 1986).

FIG. 1. Study streams of the upper Fraser region of central British Columbia. The approximate locations of the upper (U) and lower (L) spawning areas are shown for the Nechako River.

To circumvent the problem of having only spawner counts to index survival, I compared trends of the escapement of Nechako chinook with a composite trend derived from the abundance of chinook spawners from nearby unregulated streams. This statistical approach for assessing environmental impacts is relatively new (Walters 1986) and has not been used in studies of salmon populations affected by flow regulation. The method relies on the assumption that variation in mortality after residence in the natal stream will be similar for both the Nechako chinook and the aggregate of stocks from unregulated streams, so that any differences in population trends will be due to the effects specific to the Nechako River.

Another difficulty with estimating the effects of flow on Nechako chinook is that detailed data on spawner distribution and abundance are only available for recent years (after 1973), during which the annual pattern of discharge has been relatively constant. The degree of water abstraction relative to the preregulation flow is greater for the upper Nechako (above the confluence of the Nautley River; Fig. 1) than the lower river because inflows from the Nautley and Stuart rivers (Fig. 1) reduce the impact of flow reductions in the Nechako River below the confluence of the Stuart River. The difference between the upper and lower reaches is greatest in the spring months; in the upper Nechako, flows in June are only 12% of the historical average whereas below the confluence of the Stuart River, 51% of the original flow remains (Fig. 2). I tested the hypothesis that proportionately smaller flows in the upper river have had a greater impact on chinook productivity compared with further downstream, where the discharge regime is closer to the historical hydrograph (Fig. 2).

TABLE 1. Population size and life history parameters of upper Fraser chinook stocks from 1974 to 1991. Date of peak spawning from Fish Habitat Inventory and Information Program (1991). Age data are the percent composition of the most common age classes; age 1.1 and 1.4 fish usually comprise less than 10% of the total escapement. The last column is the correlation between individual escapement series and the scores of the first factor (UFI) estimated by common factor analysis. Chilko and Nechako data were not included in the UFI.

Stock	Mean escapement	Peak spawning	Age 1.2	Age 1.3	Correlation with UFI
Bowron	4123	Sept. 1	20	78	0.96
Chilcotin	1693	Sept. 5	35	65	0.88
Chilko	5924	Sept. 15	18	76	—
Cottonwood	1048	Aug. 25	63	37	0.85
Fraser	3083	Sept. 5	9	80	0.79
Holmes	1289	Aug. 20	0	89	0.97
McGregor	1613	Aug. 18	25	73	0.88
Nechako	1893	Sept. 15	24	72	—
Quesnel	2747	Sept. 20	20	70	0.85
Slim	2406	Sept. 5	34	66	0.88
Torpy	1469	Aug. 18	20	73	0.96
West Road	2600	Sept. 1	34	66	0.74
Willow	770	Aug. 25	33	66	0.92

Methods

Data Sources

Nechako chinook escapement estimates for the years 1974–83 were usually based on one or two aerial surveys (Jaremovic and Rowland 1988); in 1980 and after 1983, more surveys occurred and the area-under-curve method (Neilson and Geen 1981) was used (NFCP 1989, 1990). Redd counts were incorporated in some estimates (Jaremovic and Rowland 1988) when water turbidity impaired visual surveys of spawners. There was an increasing trend in survey effort in recent years, so I constructed two series of spawner count data: the first using only the number of fish observed on the date of the peak aerial count and the second using published estimates taken on face value. The first series employs the most consistent methodology over the whole time period, but is probably less accurate than the published estimates in the most recent years. Unless otherwise indicated, all analyses were conducted with the more consistent peak count time series.

An index of spawner abundance for chinook stocks from unregulated upper Fraser streams was constructed using factor analysis. Analysis was restricted to streams with an average of more than 1000 spawners in the 1980s. Details of the enumeration methods and an analysis of the consistency of the spawner data are provided in the Appendix.

Some life history data were also assembled for these stocks. Average timing of peak spawning for each run was from the DFO salmon escapement database (SED; Serbic 1991). With the exception of the Nechako, sampling of spawners has not been conducted for annual age compositions, so I calculated the mean age compositions from 2 or 3 yr of data from DFO files and unpublished reports. Age compositions of Nechako spawners for each year between 1974 and 1991 were from Jaremovic and Rowland (1988) and unpublished NFCP reports.

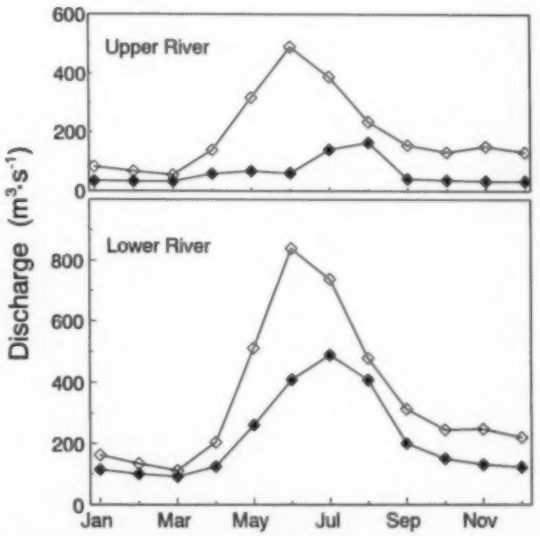

FIG. 2. Monthly mean discharges for the upper and lower Nechako. Upper river discharge is at river km 10; lower river is the sum of the upper Nechako, Stuart, and Nautley rivers. Shown are the unregulated (◇) and regulated (1974–91) average) discharges (◆).

Statistical Analysis

I first determined whether chinook spawner abundances from unregulated Fraser tributaries varied in a consistent manner, to test the assumption that stocks of the region were subject to similar sources of mortality. Common factor analysis yields a series of orthogonal factors that estimate an unobservable variable contributing to variation in the input variables (Harman 1976). Correlations between each escapement series and the factor scores provide an estimate of the

degree to which variation in mortality of a particular stock is similar to the common source of variability estimated for all stocks. Common factor analysis weights each input variable by its relative variability, so the factor scores are not necessarily dominated by large populations, as would be the case if an index of abundance was constructed from average or total escapement. I used the SAS FACTOR procedures with prior weightings set to the SMC (squared multiple correlation) option, as suggested by SAS (SAS 1988). The first factor from the analysis described the major trend common to all stocks, and scores from the first factor were used as an index of abundance for the stocks aggregate.

I calculated the relative survival of both the Nechako River chinook and those from unregulated rivers by comparing the abundance of spawners with the number of offspring returning to spawn 4 or 5 yr later. I standardized the Nechako espacement series (i.e., transformed the data so that the mean = 0, SD = 1) so that it was in the same units as factor scores from the common factor analysis of the other stocks. I then calculated relative survival, S, for each brood year t as

$$S_t = (p_{1.2}\cdot \mathrm{Esc}_{t+4} + p_{1.3}\cdot \mathrm{Esc}_{t+5}) - \mathrm{Esc}_t$$

where $p_{1.2}$ and $p_{1.3}$ are the proportions of the two major groups and Esc_t is the standardized escapement for year t. For the aggregate of upper Fraser stocks, I used an average age distribution of 25% age 1.2 and 75% age 1.3 (Table 1). All other age groups usually comprised less than 10% of the total and were ignored in the analysis. Because relative survival for the Fraser stocks was calculated from the overall trend in abundance of all stocks, S_t was an index of variation in mortality (natural and fishing) that was common to all stocks.

I used the difference between S_t values for the Nechako and the upper Fraser aggregate as an index of variation in brood survival of Nechako chinook due to the part of the life cycle not in common, i.e., the early freshwater period spent in the Nechako River. I called this measure in-river survival. This method is based on the assumption that once Nechako chinook left the Nechako River, they would experience variation in mortality similar to that of the unregulated stocks. All upper Fraser River chinook populations are "stream-type" (Fraser et al. 1982) and juveniles spend their first year rearing first in their natal stream, in the Fraser mainstem (Levings and Lauzier 1991), or in nonnatal streams (Murray and Rosenau 1989) before migrating to the sea as yearling smolts. Broods from all rivers enter the sea in the same year and will encounter similar oceanographic conditions. The majority of adults return to spawn after 3 yr in the ocean (age 1.3); smaller numbers spend 2 or 4 yr at sea (Table 1). These stocks are thought to begin their migration upstream in the Fraser River in June and July (Fraser et al. 1982), and most are probably susceptible to the same fisheries, although stock-specific data on ocean migrations and harvest are unavailable.

The distribution of chinook spawning in the Nechako varied considerably from year to year, and I was able to use this variation to test the hypothesis that survival has been poorer in the upper river where the relative degree of water abstraction was greatest. Chinook spawning is distributed over 140 km of river, from 10 km below the Kenney dam to the town of Vanderhoof (Fig. 1). Spawning is normally concentrated in either the upper 35 km of the river or in a lower area, 100–140 km from the dam; scattered spawning occurs between the two areas of concentration (Jaremovic and Rowland 1988). Spawning occurs between August and October, and fry emerge in April and May. By July, most juveniles migrate downstream from the upper river, although some overwinter in the river and emigrate the following spring (Russell et al. 1983; Nechako River Project 1987). To test whether survival was poorer when spawning was concentrated in the upper river, I correlated in-river survival with the proportion of parent spawners observed in the upper area (km 0–35; from Jaremovic and Rowland 1988 and NFCP 1989, 1990 and unpublished data for 1991).

To further investigate interannual variation of in-river survival of Nechako chinook, I examined a number of environmental variables. Because low flows and cold air temperatures may have resulted in ice damage to redds and incubating eggs during winter (Blachut 1988), I correlated survival with the Nechako River (at Vanderhoof) average discharge from November to March and the severity of winter, as indexed by the number of degree-days below zero from Vanderhoof air temperatures (Blachut 1988; Inland Waters Directorate 1991). I also used average discharge from May to July to test for effects of flow on the survival of newly emerged fry.

To investigate reasons for interannual variability in spawner distribution in the Nechako, I related the proportion of spawners in the upper river to the discharge during the period of upriver migration. I hypothesized that in years when salmon expend more energy reaching the spawning areas by swimming against stronger river flows, they are most likely to spawn in the lower reaches of the Nechako than make the additional 100-km migration to the upper area. Therefore, I calculated the correlation between the August discharge in the Nechako River at Vanderhoof and spawner distribution.

I also tested whether the distribution of chinook spawners in the river was caused by the propensity of offspring to return to the area of the Nechako River where they were hatched. For each brood, spawner distribution and age structure data were used to derive the proportion of offspring spawners using the upper river, and the correlation between the proportion of parents and offspring using the upper river was calculated. A positive relationship would suggest that adults were homing on the same reaches of the river their parents used.

Results

Population Trends in Unregulated Streams

When all of the populations from unregulated rivers were included in the factor analysis the first factor accounted for 73% of the total variance; escapements for all stocks except the Chilko River population were highly correlated with this factor (for Chilko: $r = 0.26$; for the other stocks: all $r \geq 0.74$). A second factor analysis, run with the Chilko data deleted, explained 86% of the variance in the escapement data; the second factor accounted for an additional 6% of the total variability. I called the scores of the first common factor from this run the upper Fraser index (UFI) and used it as an index of abundance for unregulated streams. Correlations between individual escapement series and the UFI were all ≥ 0.74 (Table 1).

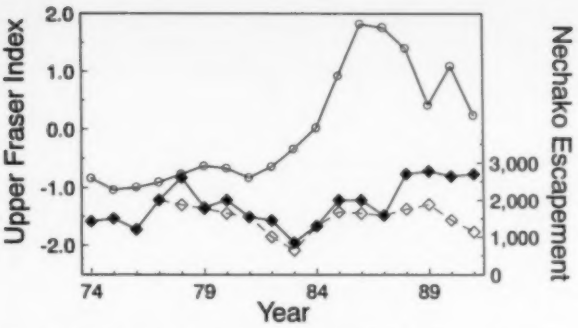

FIG. 3. Time series of published Nechako River chinook spawner abundance estimates (◆), peak spawner counts (◇), and the first factor extracted from factor analysis of UFI chinook stocks (○).

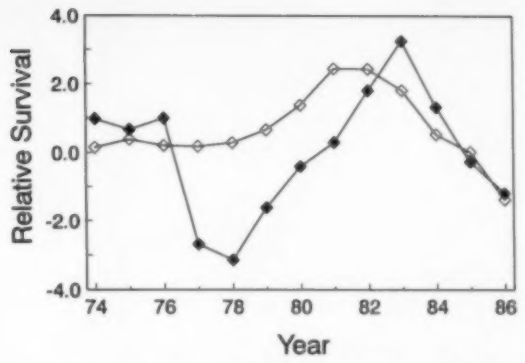

FIG. 4. Survival of Nechako (◆) and UFI (◇) chinook salmon stocks. Relative survival is the difference, in standard deviations, between the brood escapement and the returns to escapement 4 and 5 yr later. Peak spawner counts were used to derive the Nechako series.

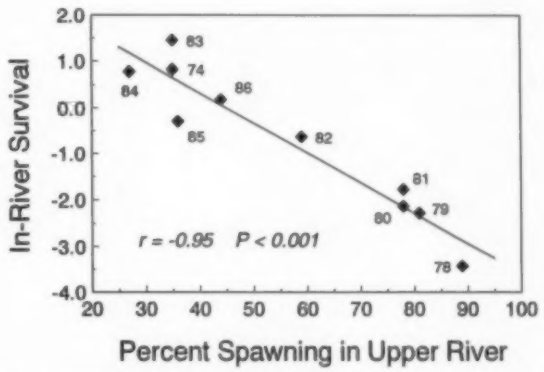

FIG. 5. Relationship between the proportion of spawners that use the upper areas of the Nechako River and the relative in-river survival of the brood (peak counts), with the year of spawning beside each point. In-river survival is the difference, in standard deviations, between Nechako and UFI brood survival from Fig. 4.

Nechako River

Nechako escapement did not increase in the 1980s as did the other upper Fraser River chinook stocks, and as a result the Nechako escapement trend was not strongly correlated with the UFI (using the Nechako peak count series: $r = 0.16$; for the published estimates: $r = 0.40$; Fig. 3). Escapement-to-escapement survival of Nechako and UFI broods was also not strongly correlated ($r = 0.20$; Fig. 4). Survival of the Nechako stock reached a minimum in 1978 and gradually increased during the 1980s, while the relative survival of the UFI stocks increased in the early 1980s. The survival of all chinook stocks has declined in more recent years (Fig. 4).

There was a strong inverse correlation between in-river survival of Nechako chinook broods and the proportion of parents using the upper Nechako River for spawning (km 0–35; for peak counts: $r = -0.95$, $P < 0.0001$, $N = 10$; Fig. 5). For the series of published escapement estimates the correlation was similar ($r = -0.90$, $P = 0.0004$). Conversely, in-river survival was positively related to the proportion of parents spawning below the Nautley confluence (km 85–140; $r = 0.85$, $P = 0.0021$). This result supports the hypothesis that survival in the upper river was poorer because flow had been reduced to a greater extent than in the lower reaches. In-river survival was not related to discharge during the incubation period (correlation using November–February discharge at Vanderhoof; $r = 0.38$, $P = 0.20$, $N = 13$). There was a weak negative correlation between in-river survival and the severity of winter, as measured as degree-days ($r = -0.53$, $P = 0.06$, $N = 13$). No relationship was found between average discharge during the April–June rearing period and survival ($r = 0.27$, $P = 0.37$, $N = 13$). Similar results were obtained using the published escapement estimates.

The proportion of chinook spawners using the upper spawning area was negatively correlated with the magnitude of flows released in August in the Nechako River (mean discharge calculated from August 1 to 20: $r = -0.67$, $P = 0.011$, $N = 13$; from August 1 to 10: $r = -0.76$, $P = 0.0025$; Fig. 6). Chinook adults tended to use the lower areas for spawning in years when large releases were made to cool the lower river for migrating sockeye salmon, but the significance of the relationship depended on three data points from the low-flow years of 1978–80.

The distribution of parents and their offspring along the length of the river was not positively correlated ($r = -0.59$, $P > 0.10$, $N = 8$). The weak and negative correlation does not support the hypothesis that offspring spawn in the same reaches as their parents.

Discussion

Population Trends in Unregulated Streams

By developing an index of survival for unregulated rivers, I was able to isolate and analyze freshwater survival of Nechako chinook, thereby overcoming the limitation of having only escapement data as a measure of population abundance. The high correlation among escapement trends of most of the chinook stocks from unregulated rivers in the upper Fraser region supports my hypothesis that these stocks have common sources of mortality. Coherence in variation in mortality might begin during incubation, if broad-scale weather patterns (such as periods of extreme cold or high precipitation) affect the subsurface gravel environment similarly in all streams in the region. Common variation in sur-

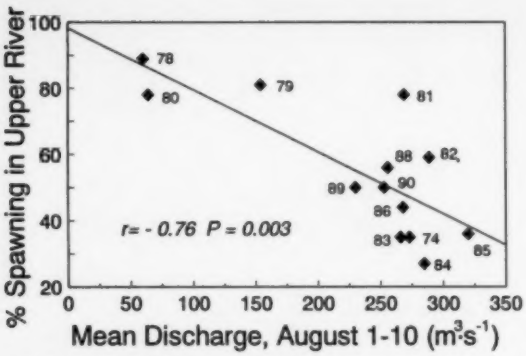

FIG. 6. Relationship between the mean discharge in the Nechako River at Vanderhoof, August 1–10, and the percentage of chinook spawners that used the upper river in that year. Discharge data from Environment Canada, Inland Waters Directorate, North Vancouver, B.C.

vival will also extend to the late freshwater and marine periods, including fishing mortality.

Differences in migration timing may explain the low correlation between Chilko River escapement and data from the other unregulated upper Fraser River stocks. Fraser et al. (1982) noted that a large chinook escapement in the Chilko River in 1975 (which contributes to the poor correlation) was due to a commercial fishing strike in early August 1975. No other stock in the upper Fraser group showed a large increase that year. Fishwheel catches at Prince George (800 km from the mouth; Fig. 1) in the early 1960s indicated migration peaks in late July and early August, suggesting that the uppermost stocks moved through the lower river earlier than the Chilko run (Chatwin et al. 1963). Chilko chinook are similar to the other stocks in age structure (Table 1) and juvenile migration behaviour (Delaney et al. 1982) so that these life history differences are not likely causes of the low correlation between this population and the UFI index.

Nechako River

With the multivariate analysis of escapement data from unregulated populations, I was able to demonstrate quantitatively that the trend in abundance of Nechako chinook has not followed that of chinook from other rivers of the upper Fraser watershed. Poor survival of chinook broods that spawned predominately in the upper Nechako River between 1977 and 1981 may be the explanation for this divergence. Returns from these broods coincided with years when reductions in fishing pressure were thought to be responsible for the increase in escapement levels observed in the UFI populations in the mid-1980s (DFO 1987). The potentially adverse effects of both extremely low flows and cold climatic conditions on egg-to-fry survival of the 1979 brood were noted by Russell et al. (1983) and Jaremovic and Rowland (1988). However, from my analysis, it appears that the freshwater survival of the 1979 brood was no worse than the others during the 1978–81 period when spawning was concentrated in the upper river (Fig. 5). The winters during this period ranged from very cold (1978) to the mildest on record (1980; Blachut 1988), suggesting that cold weather alone is not the cause of lower survival. For the most recent years (1982–86), spawning was more prevalent in the lower reaches and the trend in brood survival followed that of unregulated rivers. Variation in survival along the length of the river may explain why the effects of flow or climatic conditions, when averaged over the whole river, were not very successful in predicting survival. Correlations between the mean flow and fish survival have been found for some other regulated rivers (Rulifson and Manooch 1990; Hvidsten 1993), although in other cases, effects of flow were not detectable (Fraser et al. 1983; Nelson 1986).

Reduced survival of the 1978–82 broods was unlikely to have been due to the crowding of spawners in the upper section of the river. Spawning is spread throughout the 30 km of this reach (Jaremovic and Rowland 1988), and in 1980, a year when over 75% of spawners used the upper area, the density of redds was less than 1·100 m^{-2} (Neilson and Banford 1983). Neilson and Banford (1983) noted that redd superimposition was unlikely because redds were spaced uniformly in the spawning areas, and the long residence of females prevented late-arriving fish from digging up completed nests. They did suggest that some reduction in incubation success may have occurred if late-arriving spawners were forced to use less preferred habitats.

Poor survival of chinook broods spawning in the upper river may be due to reduced egg-to-fry survival. Blachut (1988) hypothesized that incubating eggs in the upper river are vulnerable to ice-related mortality because the river water temperature in the upper river fluctuates around 0°C in winter, and repeated freeze–thaw cycles and formation of frazil and anchor ice have been observed. Anchor ice may restrict the flow of water into the gravel and reduce oxygen concentrations in the water surrounding the eggs. In contrast, the lower river usually freezes over in early winter and surface ice persists until March or April (Blachut 1988). However, in a separate study, I planted chinook eggs in artificial redds in the upper and lower spawning areas and found no difference in mean survival over the 1992–93 winter (both were 54%; M.J. Bradford, unpublished data). Gravel permeabilities were high in both regions (>25 000 $cm \cdot h^{-1}$; M.J. Bradford, unpublished data), indicating that low survival of offspring from the upper river is not due to poorer quality spawning gravel. More data are required to adequately test the hypothesis that ice is an important determinant of year-class strength.

Early emergence of fry from the upper spawning areas may contribute to their poorer survival. Among the salmonid species the timing of emergence of fry from spawning gravel is thought to have evolved to maximize fry survival (Tallman 1986; Brannon 1987). Cramer et al. (1985) demonstrated that flow regulation in the Rogue River, Oreg., has resulted in an advancement of emergence timing and that early emergence has had a negative effect on fry survival. In the Nechako River, water temperatures in the upper river are 2–4°C warmer than in the lower area during the fall months when most embryo development occurs (DFO, unpublished data). The temperature difference exists because, in the fall, water released from the reservoir is relatively warm and is cooled by the air as it travels downstream; the rate of cooling is greater with lower flows (Blachut 1988). Using the developmental stage table of Vernier (1969), I estimated that alevins recovered from the upper river during the 1992–93 egg plant study were about 20% (180–200 degree-days) further advanced that those from the lower spawning

area (M.J. Bradford, unpublished data); these data suggest that emergence may be advanced by up to a month in the upper river. The difference in emergence timing along the length of the river would have been less before flow regulation because discharges during the incubation period were three to four times greater than they are currently (Fig. 2), resulting in more even temperatures throughout the length of the river.

After emergence, many of the fry from the upper river migrate downstream in May and June (Russell et al. 1983), when the greatest difference between the current and natural discharge occurs (Fig. 2). In unregulated rivers, fry emergence coincides with spring freshet, and chinook fry use flooded areas with vegetative cover along the stream margins and sidechannels for rearing (Healey 1991; M.J. Bradford, unpublished data). Such habitat is unavailable in the upper Nechako because the discharge rises only slightly from the winter minimum (Fig. 2) and vegetation has not yet encroached onto the exposed gravel bars. The spring freshet in an unregulated river may also serve to decrease predation during outmigration by diluting the concentration of predators and increasing the rate of passage of migrants through the river. The migration rate of juvenile salmon is positively related to discharge (Raymond 1968; Berggren and Filardo 1993), and Ginetz and Larkin (1976) demonstrated that predation rates are reduced at higher water velocities. Increases in turbidity associated with the freshet will also lower predator efficiency (Ginetz and Larkin 1976; Gregory 1993).

Nothing is known about fry migrations from the lower spawning areas. If these fry move 20–40 km downstream to rearing areas below the confluence of the Stuart River, they may benefit from increased flow and turbidity from the input of the spring freshet from the Stuart River (Fig. 2). In addition, the majority of the Stuart River chinook fry migrate to the lower Nechako in May (Lister et al. 1981) and may serve to buffer Nechako fry from predation. A recent survey of bird predators on the Nechako found that the density of the most frequently observed species, the common merganser (*Merges merganser*), was twice as high above Vanderhoof than below this point in the river, but their impact on chinook fry populations has not been documented (T.G. Brown, Pacific Biological Station, Nanaimo, BC V9R 5K6, personal communication). Predation on chinook fry by larger fish appears low (Brown et al. 1992), but these data were collected in the fall months when chinook salmon are much scarcer than in spring.

I found some evidence that the distribution of spawners in the Nechako River was affected by discharge. With the exception of 1981, use of the upper Nechako River for spawning by chinook was highest in years when there were no large cooling releases for migrating sockeye salmon (Fig. 6). An effect of water temperature on migration and chinook spawner distribution has also been observed in the Rogue River where controlled releases of cool water are made to delay upstream migration (Larson 1984). In the Nechako, the cooling releases may indeed induce spawning in the lower river, but because these releases are currently made annually, years with contrasting high and low flows needed to confirm this hypothesis are unavailable.

There remains the possibility that the chinook populations from the various spawning areas constitute genetically distinct subpopulations, as have been observed in the Nanaimo River, B.C. (Carl and Healey 1984). This seems unlikely because interbreeding would have occurred in the 1950s when low flows during the period of reservoir filling forced nearly all spawners to use the lower reaches (Jaremovic and Rowland 1988). Homing to specific spawning areas is also not supported by the parent-offspring correlation, suggesting that proximal environmental effects are more important than homing in determining spawner distribution.

In conclusion, I used a new application of multivariate statistics to show that the trend in Nechako chinook abundance has diverged from that of adjacent stocks. The divergence was due to the poor survival of broods using the upper river, where the relative degree of water removal was greatest. I was unable to isolate a single factor responsible for the poor survival because research conducted to date on the Nechako River chinook stock has not been able to identify the habitats or life stages critical to the determination of year-class strength. I propose that early emergence and a lack of a spring freshet may lower the survival of fry emerging from the upper river. If these hypotheses are confirmed, it appears likely that further reductions in flow on the Nechako will have further negative impacts on the capacity of the upper river to produce chinook salmon.

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Appendix

Here I describe the escapement dataset for unregulated upper Fraser streams and an analysis of their consistency. Chinook escapement data (1974–91) were from the SED (Serbic 1991) and N. Schubert (DFO, 610 Derwent Way, New Westminster, BC V3M 5P8, personal communication). For some streams, I summed escapements from small tributaries and the mainsteam to obtain total system estimates. These systems (with tributaries) were Bowron (Haggen, Indianpoint), McGregor (Captain, Fontoniko, Herrick, James, Seebach), Quesnel (Cariboo), and Torpy (Walker). In two cases, for tributaries of the McGregor system (James, 1985), and Fontoniko, 1990), I interpolated missing data by taking the average over the adjacent years.

Since 1986 some spawners in escapement counts originate from hatchery releases. For the Quesnel and West Road rivers, the estimated hatchery contributions were subtracted from the total escapements, but this adjustment made little difference to the results, as hatchery fish comprised only 5–15% of the total spawning population (data from C. Cross, DFO, 555 W. Hastings, Vancouver, BC V6B 5G3). There were also many small releases from the Quesnel hatchery to other systems, but returns were thought to be few (<5–10% of the escapement) and were ignored in this analysis. The Stuart River escapement series was complicated by both unmarked hatchery releases and changes in enumeration methods and was not used in the analysis. Mark–recapture techniques have replaced aerial counts since 1989 (G. Faulkner, DFO, 555 W. Hastings, Vancouver, BC V6B 5G3, personal communication); this change has probably been the cause of the doubling of abundance estimates in recent years (Simpson 1984).

The escapement data are only indexes of true spawner abundances if the method of enumeration has remained constant over time. Annual escapement estimates normally result from one or more aerial surveys of spawning grounds conducted by local fisheries officers. In some cases, information from float or streamside surveys is incorporated with the aerial counts. Counts are sometimes multiplied by subjective inflation factors to arrive at a total spawner estimate, but it is likely that the true escapements are somewhat higher than the estimates (Shardlow et al. 1987). The most precise escapement data will result from the use of the same

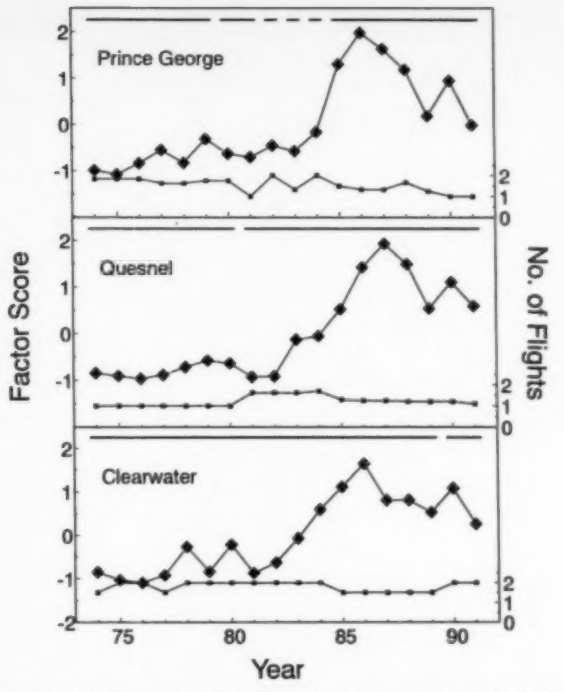

FIG. A1. Factor scores for the first factor extracted from the analysis of escapements, grouped by DFO subdistrict. Groupings are Prince George (McGregor, Slim, Torpy, Willow), Quesnel (Bowron, Chilcotin, Cottonwood, Quesnel, West Road), and Clearwater (Holmes, Fraser at Tete Jaune). The line at the top of each panel shows the intervals when enumerations were conducted by the same personnel. Exceptions are Bowron and Cottonwood, where changes occurred in 1984 and 1989. The lower line in each panel is the average number of aerial surveys conducted per stream per year.

observers and number of overflights each year; changes in personnel or effort may create artificial trends in the escapement records which would bias the results of any analysis. I examined the original data sheets and a summary of data consistency (Fielden and Slaney 1991) to determine if survey effort or personnel changes have occurred.

Responsibility for escapement estimates for the upper Fraser rivers was divided among staff of three subdistrict DFO offices (Quesnel, Prince George, and Clearwater); usually the same personnel enumerated all rivers in each district each year. I divided the rivers into three groups corresponding to their enumerating office, ran a factor analysis, and used the first factor scores as a composite escapement series for each subdistrict (Fig. A1). These indexes were highly correlated with each other (all $r \geq 0.90$) and were all strongly correlated with the first factor extracted from an analysis of all stocks (all $r \geq 0.94$). In all three series, there was a major increase in abundance in the 1980s, and for the Clearwater and Quesnel districts, no changes in personnel occurred during this period of increase. There were a number of staff changes in Prince George in the 1980s, and the relatively large increase in escapement for this region from 1984 to 1985 may have been due to a change in personnel that year. Nonetheless, the trend in the Prince George district was similar to that in the other regions, suggesting that changes in personnel were not the cause of the overall trend.

The large increase in escapements was not due to an increase in the number of overflights, which have averaged between one and two per stream during the study years. For most systems, there was a switch to using helicopters rather than airplanes around 1978–80, but there was no apparent effect of this change on escapement estimates (Fig. A1).

From this analysis, I concluded that the overall trend in the upper Fraser chinook spawner abundances (Fig. 3) was not the result of changes in methods or personnel over time and that these data were suitable for the derivation of an index of abundance for unregulated rivers.

Physiological and Growth Differences in Three Stocks of Underyearling Sockeye Salmon (*Oncorhynchus nerka*) on Early Entry into Seawater

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We compared the impact of exposure to seawater on three sockeye salmon (*Oncorhynchus nerka*) stocks: one that normally migrates to sea as underyearlings (sea-type) and two with the more common life history strategies of 1 (river-type) or 2 (lake-type) yr of freshwater residence prior to seaward migration. Innate differences in survival, ability to regulate tissue chlorides, and oxygen consumption when first introduced into salt water were more evident in April and May when fish were less than 50 mm in length. In fish longer than 50 mm, the only significant differences among the stocks were in saltwater growth. Between June and August, sea-type fish showed faster growth than river-type fish which in turn grew faster than lake-type fish. When introduced into salt water in October, virtually no growth occurred in any stock, regardless of fish size. River-type and lake-type sockeye, which normally overwinter 1 and 2 yr, respectively, in freshwater, can be reared in seawater if underyearlings are raised to a length of 50 mm before release into salt water, similar to the normal life history of sea-type underyearlings. Early life history appears to be influenced more by habitat than by genetics.

Nous avons mesuré l'impact de l'exposition à l'eau de mer de trois stocks de saumons rouges (*Oncorhynchus nerka*); le premier retourne normalement à la mer avant d'avoir un an (type marin) alors que les deux autres ont des moeurs plus courantes, soit de passer 1 an en eau douce (type lotique) ou 2 ans en eau douce (type lacustre) avant de migrer vers la mer. Les différences innées, au regard de la survie, de la capacité de régler la concentration du chlorure tissulaire et de la consommation d'oxygène, lorsque les sujets sont placés pour la première fois dans l'eau de mer, sont le plus apparentes en avril et en mai alors que le poisson mesure moins de 50 mm de longueur. Chez les sujets plus grands que 50 mm, les seules différences significatives entre les stocks sont observées au niveau de la croissance dans l'eau de mer. Entre juin et août, le stock de type marin grandit plus vite que le type lotique, et celui-ci grandit plus vite que le type lacustre. Lorsqu'ils ont été placés dans l'eau de mer en octobre, aucun des stocks n'a pratiquement eu de croissance, peu importe la taille des sujets. Le saumon rouge du type lotique et du type lacustre, qui passe normalement un ou deux hivers en eau douce, peut être élevé en eau de mer si les jeunes de moins d'un an sont gardés en eau douce jusqu'à ce qu'ils atteignent 50 mm avant de les transférer en eau de mer, ce qui s'apparente au cycle biologique normal des sujets de moins d'un an du type marin. Il semble que les premiers stades évolutifs sont davantage influencés par l'habitat que par des facteurs génétiques.

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After emergence, sockeye salmon (*Oncorhynchus nerka*) typically spend 1 or more yr in a freshwater lake before migrating to sea as smolts ("lake-type"). In Alaska, there are eight streams where more than 50% of the population of sockeye migrate to sea as underyearlings (McPherson and McGregor 1986). These sockeye are termed "sea-type". These fish do not produce a freshwater annulus on their scales and do not spend time in freshwater past emergence. Some sockeye populations spend 1 yr in rivers after emergence and are termed "river-type" (Wood et al. 1987). Several streams in Alaska, such as the Taku and Stikine, produce significant percentages of all three types (McPherson 1987). It is not known whether sea-type stocks have an innate capability for early entry into seawater.

Although sea-type sockeye were identified in the Fraser River system as early as 1918 (Gilbert 1918), this life his-

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tory strategy was considered rare and insignificant because of the large production from the lake systems of the Fraser River (Foerster 1968). In the mid- and late 1980s, however observations of sockeye in nonlake systems in Alaska, along with scale analyses for stock separation in commercial fisheries, demonstrated that sea-type stocks are widespread and, in some streams, the most numerous (Pahlke and Riffe 1988).

Survival and physiological capabilities of juvenile sockeye from sea-type stocks have not been compared with classic lake-type stocks. The smoltification process in salmonids, including sockeye, has received considerable attention in the past (see review by Hoar 1988; Thorpe et al. 1985). These studies, however, are dominated by research on populations from British Columbia and the Pacific Northwest where sea-type stocks are rare. Studies of out-migrating sockeye underyearlings in the Situk and Taku rivers have documented timing of movements and size of sea-type underyearlings during their first summer and their tolerance to seawater after growing to at least 50 mm in length (Heifetz et al. 1989; Murphy et al. 1991).

Smoltification and salinity tolerance in sockeye are strongly influenced by photoperiod and size (Clarke et al. 1978; Foote et al. 1992). The ability to tolerate salt water increases rapidly in the spring and tolerance increases with size. Most previous research has dealt with the effects of photoperiod and/or temperature on the adaptation of sockeye smolts greater than 70 mm in length to seawater. In Alaska, both photoperiod and temperature changes are quite large in the spring and growth is often rapid. It is not known if underyearlings (30–70 mm) with lake- or river-type histories are as capable of surviving and growing in seawater as similar-sized sea-type underyearlings.

Our primary objective was to compare three stocks of Alaskan sockeye with different life histories to determine if sea-type stocks had enhanced capability to tolerate exposure to seawater. For this comparison, we chose all three stocks from southeast Alaska (similar latitude):

(1) "Lake-type", Auke Lake — Smolts migrate to sea in May–June with two freshwater annuli on their scales and at a mean length greater than 70 mm (Bucaria 1968; Taylor et al. 1992).

(2) "River-type", Taku River (Yehring Creek tributary) — Smolts migrate to sea in May–June with one freshwater annulus after rearing in off-channel beaver ponds and sloughs and at a mean length of 63 mm (Murphy et al. 1988).

(3) "Sea-type", East Alsek River (a former outlet of the Alsek River) — Smolts migrate to sea in the first summer (no freshwater annulus) at unknown size and timing. Nearly all returning East Alsek River spawners (98%) lack a freshwater annulus (McBride and Brogle 1983).

To compare the capabilities of the three stocks, we challenged underyearlings from each stock in a series of seawater challenge tests (Clarke et al. 1978) during the spring and summer. Specifically, (1) survival was measured in a series of short-term seawater challenge tests, (2) osmocompetence, which we define as hypoosmoregulatory ability, was measured by analysis of tissue chloride after short-term seawater challenges, (3) stress and energy expenditure were monitored by measuring oxygen consumption during conversion from freshwater to seawater, and (4) growth was meaured in a series of long-term seawater challenges. We ensured the comparability among the stocks by running all tests with underyearlings of equal size from all three stocks at the same time, photoperiod, and temperature. These data will provide insight into the ability (plasticity) of wild stocks to cope with sudden environmental changes in habitat and will provide information to fish managers if they need to choose stocks of sockeye salmon for intensive hatchery culture.

Methods

Sockeye underyearlings were obtained from artificially spawned eggs from three systems in southeast Alaska. Eggs from lake-type sockeye were obtained from Auke Creek, from fish with two freshwater annuli (over 75% of returning Auke Creek sockeye spent 2 yr in freshwater; 25% spent 1 yr (McPherson and McGregor 1986)). Eggs from river-type sockeye were obtained from the Yehring Creek tributary of the Taku River from fish with one freshwater annulus (86–93% of the sockeye returning to Yehring Creek spent 1 yr in freshwater; the remainder went directly to sea following emergence). Eggs from sea-type sockeye were obtained from the East Alsek River near Yakutat, from fish without a freshwater annulus (less than 5% of the returning East Alsek River sockeye spent 1 yr in freshwater (McGregor 1986)).

Spawn was collected from 16 adults of each stock. Each adult was screened for bacterial kidney disease and IHN virus. For each stock, eggs from one adult female were mixed with milt from a single male and incubated separately. If either the male or female from the pair showed high titers of bacteria or virus, the eggs in that tray were destroyed. The eggs were incubated at the Auke Bay Laboratory in filtered water irradiated with ultraviolet light. The lake-type stock was spawned at a hatchery on 11 August 1987 and reared at ambient temperatures until transfer to our laboratory on 10 October 1987. The eggs had received 640 temperature units of rearing and were subsequently held at 4°C. The river-type stock was spawned on 23 September 1987 and incubated at 8°C. The sea-type stock was spawned on 20 October 1987 and incubated at 11°C. The differences in rearing temperatures allowed us to control the rate of yolk absorption. All three stocks of fish reached yolk absorption and were ponded on 19 January 1988. There were no differences in weight among the three stocks of newly emergent fry.

About 10 000 underyearlings of each stock were placed in separate, flow-through, outdoor aquaria (800 L) between 19 January and 23 February 1988. The aquaria were supplied with freshwater (filtered and irradiated) and maintained at ambient light and temperature (4–7°C). Fish were fed ad libitum with commercial pellets during the first month, but the amount of food was adjusted weekly to ensure that the size differences between stocks were minimized. It was important that the fish were matched in size for all tests. Numbers of fish in the aquaria declined steadily through the study period as fish were removed for testing. This had the effect of maintaining a steady density (grams per litre) in the holding tank.

Salinity exposures were created by mixing seawater (30 ppt) and freshwater (0 ppt) to supply a flow rate of 500 mL/min to 80-L flow-through aquaria (66 × 43 × 28 cm). The ratio of seawater to freshwater varied among aquaria, resulting in nine aquaria with salinities of 22, 23, 24, 25, 26, 27, 28, 29, and 30 ppt, respectively. Salinity in each aquarium was measured daily with a calibrated hand-held

TABLE 1. Length (mm), percent survival in 30 ppt seawater (96 h), and 96-h LC50 (ppt salinity) (95% confidence interval in parentheses) of each stock of sockeye underyearlings on different dates.

Stock	Calendar day			
	18 March	18 April	12 May	30 May
Auke Creek (lake-type)				
Length (mm)	35	38	42	52
% survival	0	0	15	100
LC50 (ppt)	27.7 (0.74)	28.6 (0.15)	28.3 (0.59)	>30
Taku River (river-type)				
Length (mm)	33	37	44	49
% survival	0	0	60	100
LC50 (ppt)	26.2 (0.76)	28.3 (1.91)	30.5 (0.60)	>30
East Alsek River (sea-type)				
Length (mm)	30	31	39	49
% survival	0	60	20	100
LC50 (ppt)	28.2 (0.43)	31.1 (1.13)	29.2 (0.54)	>30

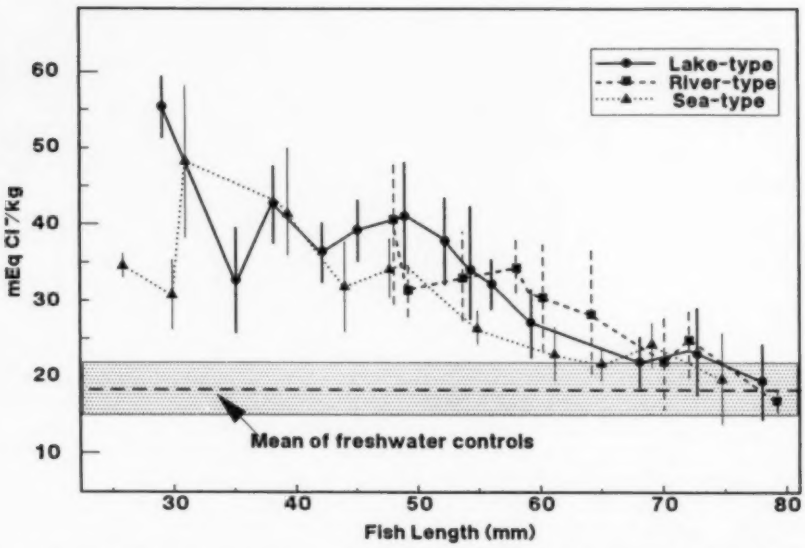

FIG. 1. Tissue chloride concentrations in three stocks of sockeye in freshwater and 24 h after transfer to seawater. Vertical lines indicate 95% confidence intervals; the shaded area indicates the 95% confidence intervals for freshwater controls.

salinometer. Each tank was stocked with 20 fish from each stock for the salinity bioassays and 100 fish from each stock for tissue chloride determinations. Stocks were separated in each tank by placing them in perforated (4.8-mm holes) polyethylene containers (25 cm^3).

Salinity tolerance was determined by 96-h flow-through bioassays every 2 wk between February and October. Fish were exposed to nine salinities ranging from 22 to 30 ppt. Survival was determined daily, and dead fish were removed daily. Lethal median concentrations (96-h LC50 were determined for each stock from mortality data by logit analysis (Silverstone 1957).

Tissue chloride concentrations in seawater-exposed fish were determined at the same time as the bioassays. Tissue chloride levels were determined by the method of Barron (1986) rather than determining traditional plasma sodium levels (adequate plasma volume was not available in small underyearlings). Ten fish of each stock were removed from a 30-ppt tank at 0, 6, 12, 18, 24, 30, 36, and 48 h of exposure. Eviscerated fish were beheaded, weighed, and homogenized in 10% perchloric acid to precipitate large molecular weight compounds. The supernatant from the centrifuged homogeneate was analyzed with an amino chloride titrator. Tissue chloride concentrations were recorded as milliequivalents of chloride per kilogram of eviscerated fish. Biweekly means were compared by Student's *t*-test.

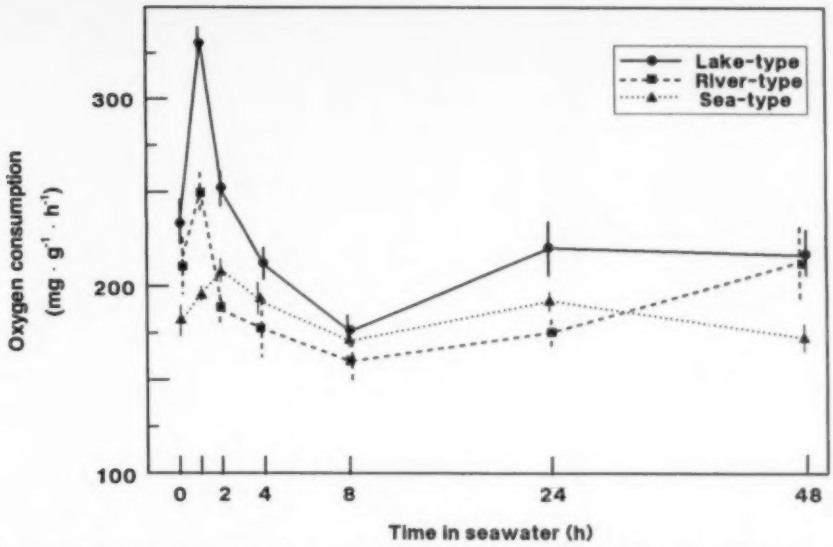

FIG. 2. Oxygen consumption of three stocks of sockeye before and after transfer to seawater. Vertical lines indicate 95% confidence intervals.

Oxygen consumption was determined on 60- to 65-mm fish from each stock in 9 × 4.5 cm cylinders constructed from plastic pipe. Water flow through the chambers was maintained at a level (100–140 mL/min) at which fish could reduce the partial pressure of oxygen of the chamber water approximately 30–40%. Oxygen partial pressure of the water flowing through the respiration chambers was determined with a Radiometer PO_2 electrode coupled with a PHM71 Mk2 acid– base analyzer fitted with a PHA934 oxygen module. Single fish from freshwater rearing tanks were placed into 20 separate chambers and allowed to acclimate for 40 h before oxygen consumption was determined. This permitted measurement of resting levels for each stock. Observations were made to ensure that data were taken only from quiescent fish. All measurements were made on fish 60–65 mm in length at an ambient water temperature of 8.3°C (±0.1°C). Following measurement of oxygen consumption in freshwater (control values), 15 of the chambers were converted to seawater (30 ppt) and oxygen was measured at 1, 2, 4, 8, 24, and 48 h postconversion. Five chambers were retained on freshwater and served as controls. Differences in mean oxygen consumption were tested at the 0.05 level by analysis of variance (Sokal and Rohlf 1969).

Growth in seawater was determined monthly. Growth tests were started on 1 June when the mean length of all stocks was at least 50 mm and fish could survive 30 ppt seawater. On the first day of each month, 100 fish from each stock were transferred to compartments of a Living Stream aquarium with a seawater flow rate of 2 L/min and held for 30 d. Temperature was held at 8°C in all growth tanks, regardless of ambient temperature. All three stocks had similar mean lengths at the beginning of the growth tests. Changes in length and weight were measured over 30 d. Instantaneous growth rate was calculated by

$$R = \frac{\ln \frac{F_2}{F_1}}{t}$$

where R is the instantaneous growth rate, t is the testing interval in days, F_2 is the final size, and F_1 is the initial size. Standard error of the regression of the growth curve was used to determine the 95% confidence invervals.

Results

Survival of the three stocks in seawater varied initially, but after fish grew to about 50 mm, all had 100% survival (Table 1). Sea-type sockeye were initially more tolerant of seawater than the other two stocks; these fish had greater LC50s and were the only stocks to have some survival in 30 ppt seawater after 96-h exposures at 30–39 mm. By the time the fish had reached 50–55 mm, all three stocks were capable of surviving a 96-h exposure to 30 ppt seawater. Although sea-type fish had slightly higher LC50s than the other stocks in the early tests, differences were not statistically significant.

Fish in the 30- to 50-mm size range showed more variability in seawater tolerance than the larger fish. The 30- to 50-mm size range was the transition zone between partial tolerance and full tolerance. Individual tolerance was influenced by a combination of factors, including size, temperature, condition, and perhaps stock.

During the early sampling periods, all stocks exposed to seawater for 24 h had tissue chloride concentrations that were significantly greater than their freshwater counterparts (Fig. 1). Lake-type underyearlings at 29 mm had the highest concentration measured (55 mEq Cl^-/kg) in the few fish that were able to survive until the 24-h sampling time; there were no survivors at 96 h. In contrast, control fish in freshwater had a mean of 17.3 mEq Cl^-/kg (95% confidence interval of 15–21 mEq Cl^-/kg); there were no differences among stocks. Sea-type fish at the first sampling indicated some ability to osmoregulate during the first 24 h because their tissue chloride concentrations (34.9 mEq Cl^-/kg) were significantly ($P < 0.05$) less than those of lake-type underyearlings, even though their survival at 96 h was similar.

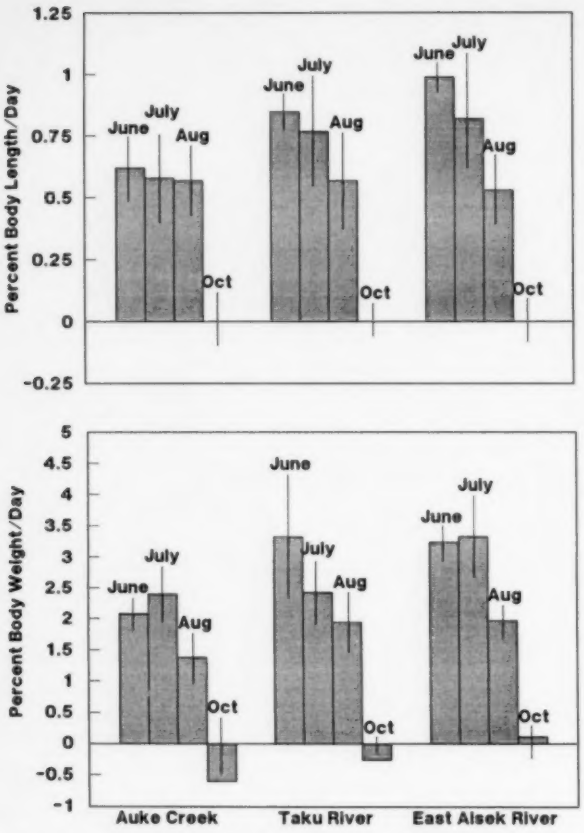

FIG. 3. Instantaneous growth rate (length and weight) of three stocks of sockeye during the first 30 d after entry into seawater. The first day of entry was the first day of the month for each testing period. Vertical lines indicate 95% confidence intervals. Auke Creek = lake-type; Taku River = river-type; East Alsek River = sea-type.

As fish grew, tissue chloride concentrations measured after 24 h of seawater exposure declined. Above 60 mm in length, tissue chloride concentrations for the three stocks overlapped each other and control values.

Oxygen consumption increased for all three stocks in the first hour after conversion of the respiration chambers from freshwater to seawater (Fig. 2). The sea-type stock increased consumption by 15%, river-type by 19%, and lake-type by 42% after 1 h in seawater. The sea-type stock had the lowest control value (freshwater consumption) of the three groups; it was significantly ($P < 0.05$) lower than the other two. Oxygen consumption of the river-type and lake-type stocks peaked at 1 h following conversion to seawater, and both groups were at or below control levels 2 h post-conversion. Sea-type fish reached maximum consumption by 2 h and were back to control values at 4 h. All stocks were still at control levels after 48 h in seawater. Oxygen consumption of sea-type fish was significantly ($P < 0.05$) less than of lake-type fish at all recording periods and was significantly less than both stocks at 48 h.

Metabolism was under greater stress for the lake-type stock and least for the sea-type stock when transferred into seawater, even though all stocks were of sufficient size (60–65 mm length) to survive and grow in seawater. Sea-type fish had the lowest resting oxygen consumption in freshwater and also exhibited the least increase in uptake upon conversion to seawater. River-type fish had only a slightly greater percent increase in oxygen consumption than did sea-type fish, while lake-type fish increased oxygen consumption by more than two times that of the other two stocks.

All three stocks survived for 30 d and grew in seawater, but sea-type sockeye grew faster than the other three stocks when first exposed to seawater in June (Fig. 3). In June, sea-type fish grew about twice as fast in length (1% body length/d) as lake-type fish (0.65% body length/d) ($P < 0.05$), but growth in other summer months was not significantly different. Growth of river-type fish in June was intermediate between lake-type and sea-type fish. As summer progressed, fish from all three stocks were larger on first exposure to seawater, and the differences in growth between the three stocks disappeared. First exposure of fish to seawater in October resulted in negative weight gain, even though the fish were larger (80 mm) than their summer cohorts (50–70 mm) which grew well in seawater (Fig. 3). Day length decreased between June and October, but the test temperature was always 8°C. The October growth test began 10 d past the autumnal equinox (when daylight was 11 h), but the holding temperature was above 7°C and test temperature was at 8°C, as in all growth tests.

Discussion

The results of our laboratory seawater tolerance tests are consistent with field tests on Situk River sockeye; sea-type fish survived seawater challenge tests after achieving a length of 50 mm (Heifetz et al. 1989). Sea-type sockeye evidently accomplish this growth either by migrating soon after emergence to an estuary, where the salinity is typically 26–28 ppt (Heifetz et al. 1989), or by inhabiting riverine sloughs and ponds for 2–3 mo before migrating to sea in June and July at a length of 50–70 mm (Birtwell et al. 1987; Wood et al. 1987; Murphy et al. 1988). The ability of sockeye underyearlings to survive in seawater may be an adaptation in fish living in freshwater river systems where lake habitat is limited, but where estuarine conditions are accessible and allow underyearlings to grow large enough to survive in seawater as underyearlings (Heifetz et al. 1989).

Tissue chloride concentrations reflect the ability of fish to osmoregulate in seawater — the higher the concentration, the more impaired the ability to maintain osmotic balance (Barron 1986). As fish grew, tissue chloride concentations peaked at increasingly lower levels in all three stocks when exposed to seawater. Therefore, larger fish were better able to osmoregulate and survive in seawater. Initially, based on tissue chloride concentrations, the lake-type fish were not able to osmoregulate as well as sea-type fish, and thus their survival in seawater was lower. After all three stocks reached 50 mm in length, all were able to osmoregulate sufficiently to survive in seawater. The consistently higher tissue chloride levels in lake-type fish compared with sea-type fish indicate that lake-type fish were not quite as successful in maintaining osmocompetence.

Changes in oxygen consumption after transfer to seawater reflect, first, the stress of the change in environment and,

second, changes in metabolic energy demands. The reaction to stress is obvious because of the spike in oxygen consumption. In contrast, changes in resting rates are much more subtle and difficult to evaluate statistically. The lake-type stock was definitely more stressed after exposure to seawater, even though seawater tolerance was within their physiological capabilities. Stress can be defined as diversion of metabolic energy from an animal's normal activities (Barton and Schreck 1987). For example, a 10 and 20% increase in respiration rate was projected to decrease growth of coho salmon (*Oncorhynchus kisutch*) 8 and 16%, respectively (Vaughan et al. 1982). A lower increase in oxygen consumption upon transfer to seawater indicates an ability to adapt to the new salinity with lower energy expenditure and, therefore, less stress. As indicated by Zimmerman (1965), the economical utilization of energy is intimately associated with adaptation to a particular environment, and the bioenergetics of a species is a basic determinant of its niche requirements. On this basis, the sea-type and river-type underyearlings seem better suited for early migration to seawater.

The inability of fish to grow in seawater in October may be due to seasonal changes in endogenous rhythms. Our data suggest that decreases in daylight after the autumnal equinox may trigger reductions in growth. Underyearling sockeye salmon have been shown to undergo a seasonal cycle in their ability to regulate plasma sodium concentrations during 24-h seawater challenge, with declining ability in fall and winter (Foote et al. 1992). The ability to regulate serum sodium concentration can be altered by artificially extending the photoperiod (Clarke et al. 1978).

There were stock differences during the tests. Sea-type fish appeared to have some early survival advantages before they reached 50 mm in length. Growth after first exposure of sea-type fish into seawater in June, when they were just past 50 mm in length, was significantly greater than growth of lake-type fish. Oxygen consumption, as a measure of stress during entry into seawater, indicates that sea-type fish were subjected to less stress than lake-type and river-type fish. The changes in metabolism, as measured by oxygen consumption, mirror the results observed with studies of survival, growth, and tissue chloride concentrations upon transfer of the three different stocks to seawater. The ability to survive and grow in seawater requires the ability to osmoregulate. The greater the amount of energy required to regulate in a hyperosmotic environment, the lower the survival rate will be, and if the fish do survive, the growth rate will be less. Sea-type fish required less energy to osmoregulate than the other two stocks and initially had higher survival and faster growth.

Stock differences in all tests disappeared as fish grew during mid- to late summer. In nature, sea-type sockeye appear to grow unusually fast, attaining a size similar to lake type smolts by July of their first summer (Birtwell et al. 1987; Heifetz et al. 1989). The rapid growth of sea-type sockeye probably stimulates them to migrate to sea in their first summer. Fish culturists can overcome stock differences if they grow underyearlings past the minimal size for release (50 mm). Acquiring a natural sea-type stock may have some early growth advantages in seawater, but is not an absolute requirement which would force the relocation of stocks. For example, 20 000 Auke Creek (lake-type) underyearlings were released from Auke Creek Hatchery in June 1988 as sea-type. These fish were held in seawater net pens for 3 wk before release at a mean length of 84 mm. The returning 2-yr and 3-yr adults from the sea-type smolt release had significantly greater ocean survival and growth than lake-type releases (Taylor and Heard 1992).

The potential application of sea-type life history strategies to aquaculture operations has renewed interest in large-scale operations with sockeye (Amend 1987). Sockeye are a premium salmon with high commercial value, but hatchery enhancement has been slow to develop because of vulnerability of sockeye to IHN virus and because rearing underyearlings in freshwater for a year past emergence is costly and risky. Release of underyearlings about 2 mo after emergence would reduce hatchery costs and risks (total holding time is cut in half, and only one year-class of sockeye would have to be in the hatchery at any one time). Sockeye stocks with other life histories can be adapted in a hatchery to this sea-type life history.

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LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Comment on "Genetic, Environmental, and Interaction Effects on Growth and Stress Response of Chinook Salmon (*Oncorhynchus tshawytscha*) Fry" by Heath et al. (1993)

In a recent article in the *Journal*, "Genetic, environmental, and interaction effects on growth and stress response of chinook salmon (*Oncorhynchus tshawytscha*) fry", Heath et al. (1993) made two basic errors in their analyses that undermine the conclusions drawn from their results. They compare the response to stress among progeny from several full- and half-sib families. Each family was divided in half at fertilization and incubated at two different temperatures. Parameters measured were size, growth rate, and a suite of variables to monitor the fish's response to stress. The stated objectives of this research were to investigate genetic effects (differences between families), environmental effects (differences between groups incubated at different temperatures), and genetic by environmental interactions (i.e., changes in family ranking at the different incubation temperatures) on growth- and stress-related traits.

The first fundamental error is made in the *F*-tests for determining significance. In the description of analyses, Model A, a linear model incorporating genetic and environmental sources of variation, is given. Table 2 in the article provides the mean square (MS) values for each source of variation in Model A with each of the parameters measured and displays the significance values. The nested mating design and analytical model used indicate that significance of the dam term should be tested by the ratio MS_{dam}/MS_{sire}. However, from the significance values shown in table 2, it appears that the ratio MS_{dam}/MS_{error} was used. The test used incorrectly identifies the dam effect as significant for most of the parameters measured (only wet weight is significant with the proper test). The tests of sire and incubation temperature effects were done appropriately, yet the dam by incubation temperature interaction effect was also incorrectly tested. The proper *F*-test in this case was $MS_{dam \times temp}/MS_{sire \times temp}$.

The second major error was made in the genetic interpretation applied to the variance components. The dam component was reported to consist of $0.25V_A + V_{CE}$, and the sire component to consist of $0.25V_A + 0.25V_D$. As stated by Heath et al., the maternal effect of egg size on the progeny is indeed a common environmental effect for half-sibs; however, in their analysis they neglect to consider the common environmental effects of full-sibs being reared in the same tank through May 24. A more appropriate interpretation of the dam and sire components of variance would be $\sigma^2_{dam} = 0.25V_A + V_M$, where V_M = variance due to maternal effects, and $\sigma^2_{sire} = 0.25V_A + 0.25V_D + V_{CE}$, where V_{CE} = variance due to common environmental effects (including tank effects) (Falconer 1981).

The reason for pointing out these mistakes is that they throw serious doubt on these results as evidence for genetic effects and for genetic by environment interaction effects in the stress response of this experimental population of fish. Even in the absence of significant dam effects, one may feel that the significance of the sire effect demonstrates genetic differences. However, because of the common environment for full-sibs (sire groups) for seven of the eight months between fertilization and the start of the stress experiments, I feel that the tank effects cannot be dismissed as an unimportant factor in differences between full-sib families. Certainly, genetic effects should not be disregarded, but this study does not provide convincing evidence for a genetic effect. In the absence of demonstrated genetic effects, genetic by an environment effects cannot be credibly demonstrated. By the same reasoning as above, the changes in rank of full-sib families at different temperatures may be the result of rearing history differences, not genetic differences.

The results concerning the reduced variability among family members and the suggestion to use sib groups for higher sensitivity in stress response experiments are not challenged by these errors and I feel that this article has convincingly shown that environmental effects and specifically rearing history can influence stress response. Nevertheless the genetic analyses are unsound and led to an unfortunate misinterpretation of the results — Jeffrey T. Silverstein, *National Research Institute of Aquaculture, Nansei, Mie, Japan 516-01.* (JC089)

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Reply to Comment on "Genetic, Environmental, and Interaction Effects on Growth and Stress Response of Chinook Salmon (*Oncorhynchus tshawytscha*) Fry" by Silverstein

The primary purpose of the research described in Heath et al. (1993) was to test for genetic contributions to stress- and growth-related variables in chinook salmon fry. There is considerable interest in the physiological and endocrinological stress response of juvenile salmonids, yet there is little information concerning possible genetic involvement. Evidence for a strong genetic component to stress-related traits would alter the experimental designs used by many stress physiologists and have a major impact on the breeding plans and goals of the aquaculture industry.

In his comment, Silverstein correctly pointed out that two of the terms in the ANOVA, Model A, presented in Heath et al. (1993) were incorrectly tested for significance. Silverstein noted that the dam main effect should be tested using $MS_{S\{D\}}$ (where S{D} is sire nested within dam) as

TABLE 1. Corrected ANOVA table for growth- and stress-related traits measured on eight full-sib families of chinook salmon fry. Sire (S) and dam (D) effects are random with S nested within D, and incubation temperature (IT) is a fixed effect. Wt is wet weight, RG is relative growth, Ct and ΔCt are resting and poststress differential plasma cortisol concentrations, Gl and ΔGl are resting and poststress differential plasma glucose concentrations, and H_C and H_S are the control and poststress hematocrits. The actual mean squares are given in Heath et al. (1993). Statistical significance: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS = not significant at $P < 0.05$.

		Statistical significance							
Source	F ratio	Wt	RG	Ct	Gl	H_C	ΔCt	ΔGl	H_S
IT	$MS_{IT}/MS_{IT \times D}$	***	**	NS	NS	NS	NS	NS	NS
D	MS_D/MS^a_{comp}	**	NS	NS	NS	NS	NS	NS	*
S (S{D})	$MS_{S\{D\}}/MS_{IT \times S\{D\}}$	**	***	***	***	**	***	*	***
IT × D	$MS_{IT \times D}/MS_{IT \times S\{D\}}$	NS	NS	NS	NS	NS	*	*	NS
IT × S{D}	$MS_{IT \times S\{D\}}/MSE$	*	**	***	NS	*	NS	NS	***
Error	MSE								

$^aMS_{comp} = MS_{S\{D\}} + MS_{IT \times D} - MS_{IT \times S\{D\}}$.

the denominator in the F ratio and that the dam-by-incubation temperature (IT) interaction should be tested using $MS_{IT \times S\{D\}}$ (where IT × S{D} is the sire-by-IT interaction) as the denominator. Although we agree with Silverstein that the use of MSE as the denominator in the F ratio was incorrect, we believe that the proper denominator for testing the dam main effect is a composite error term (Table 1) with six degrees of freedom (Satterthwaite 1946). This composite error term is necessary, since our design is a moderately unbalanced mixed-model ANOVA (see Ayers and Thomas 1990; Rawson and Hilbish 1991). Table 1 gives the proper F ratios and corrected significance levels for all of the traits measured in Heath et al. (1993).

The principal conclusion made in Heath et al. (1993) was that strong genetic effects were present for all of the variables measured. Although the corrected ANOVA table (Table 1) shows fewer significant dam main effects than reported in Heath et al. (1993), the sire main effects are still highly significant. However, as Silverstein pointed out, the sire variance component is confounded with common environment (V_{CE}), or "tank" effects, since the full-sib families were held in separate and unreplicated groups during incubation and early rearing. The confounding tank effects can be corrected for, since the sires were replicated across the two incubation temperatures, and the incubation temperature effect was not significant for the stress-related traits (Table 1). We reanalyzed the stress-related data using the two environments as replicates and found significant sire main effects for all of the stress-related variables ($P < 0.05$).

In theory, genetic effects should be reflected in dam as well as sire main effects, yet in our study, the dam main effects were generally nonsignificant (Table 1). It is important to note, however, that the experimental design used in Heath et al. (1993) (eight sires nested within four dams) was far more sensitive to sire effects than dam effects. In order to quantify this, we performed power analyses of our experimental design (SOLO Statistical Systems; Power Analysis, BMPD Statistical Software Inc., Los Angeles, Calif.). We found that the power ($1 - \beta$) of the dam component in the analysis was 5–10 times lower than that of the sire component (e.g., for wet weight, $dam_{power} = 0.096$; $sire_{power} = 0.82$). Therefore, it is not surprising that few of the dam main effects were significant.

Silverstein also noted that potential tank effects would directly contribute to family-by-IT interaction effects. This is also the case for the sire(nested within dam)-by-IT interaction effects. Dam-by-IT interactions, however, would be unaffected by tank effects, since dam half-sib families were replicated within each IT. Two of the stress-related traits had significant dam-by-IT interactions (Table 1), and thus, technically, only those two traits unambiguously exhibit genotype-by-environment interactions.

Tank effects are well documented for size- and growth-related traits in salmonids (Aulstad et al. 1972; McKay et al. 1984; deMarch 1991) but have not been reported for the stress-related traits measured in Heath et al. (1993). Size- and growth-related tank effects in juvenile salmonids have been found to be correlated with fish density (deMarch 1991). However, there were no significant correlations between fish density and any of the measured traits in Heath et al. (1993) (regression analysis on the means, $P > 0.10$; $N = 16$).

Microenvironmental differences may have an impact on the growth (and thus size) of fish held in separate rearing tanks (McKay et al. 1984) and these effects may persist after the fish are transferred to a common environment. However, it is unlikely that such effects would persist for over 5 wk (the duration of experiment in Heath et al. 1993), especially for the stress-related traits. Generally, fish used in stress experiments are acclimated for less than 3 wk (Casillas and Smith 1977; Schreck 1981; Barton et al. 1986; Sumpter et al. 1986). The acute stress response of plasma cortisol concentration has been shown to last 3 h or less in juvenile chinook salmon (Barton et al. 1986) and less than 10 d in juvenile salmonids under chronic stress (Schreck 1981). It is unlikely, therefore, that the separate early rearing of the full-sib families described in Heath et al. (1993) contributed appreciably to the observed genotype-by-environment interactions for the stress-related traits after more than 5 wk in a common tank.

If our resources had allowed replication of the full-sib families within each environment, we could have explicitly tested for tank effects. Since we were unable to expand our experimental design, we did attempt to minimize potential tank effects. We feel that the magnitude of the tank effects was small for most of the measured traits, although the possibility of large tank effects cannot be formally excluded.

Silverstein has pointed out errors in the statistical analysis presented in Heath et al. (1993) and has quite correctly

questioned the conclusions concerning genotype-by-environment interactions. However, the main goal of the research described in Heath et al. (1993) was to test for genetic contributions to the measured traits. We feel that the reanalysis described here shows that the data presented by Heath et al. (1993) indicated a significant genetic contribution to most of the measured traits. These results show that the genetic and environmental background of the fish can have a significant effect on physiological measures of stress response in juvenile chinook salmon. Genetic and environmental factors should thus be taken into account by stress physiologists and aquaculturists in the future — Daniel D. Heath, *Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA*, Nicholas J. Bernier, *Department of Animal Sciences and the Canadian Bacterial Diseases Network, University of British Columbia, 2357 Main Mall, Suite 248, Vancouver, BC V6T 2A2, Canada*, John W. Heath, *Yellow Island Aquaculture Ltd., 1681 Brook Cres., Campbell River, BC V9W 6K9, Canada*, and George K. Iwama, *Department of Animal Sciences and the Canadian Bacterial Diseases Network, University of British Columbia, 2357 Main Mall, Suite 248, Vancouver, BC V6T 2A2.*

(JC149)

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OBITUARY/NÉCROLOGIE

Gerard J. FitzGerald

1949–1994

Gerry FitzGerald passed away peacefully at his home on Monday, March 14, 1994, after a nine-month battle with cancer. An expert in the behavioral ecology of fishes, he was at the height of his career when diagnosed with the disease that killed him. He was 44 years old.

Gerry was born September 19, 1949, in Newfoundland. After completing his Bachelor's degree at Memorial University, he went on to obtain his M.Sc. at Macdonald College of McGill University (1972) and his Ph.D. at the University of Western Ontario (1976) in the laboratory of Miles Keenleyside. Upon graduation, he immediately was employed by Université Laval. He obtained the status of full professor in recognition of his contributions to science and the training of graduate students in 1985.

The rapidity with which Gerry moved through both his education and career pathways was the result of his bright intellect, unbounded energy, and uncompromising determination to succeed. Gerry's science was about sticklebacks. It is a gross understatement to say that Gerry studied sticklebacks. Gerry was fascinated by them and their unlimited potential as models to test numerous hypotheses of behavioral ecology. His famous "God is a stickleback" T-shirt, Gerry's trademark during much of the 1980s, reflected his consuming passion for the fishes. This passion resulted in the publication of close to 100 articles about various aspects of their biology and behavior. Gerry, his students, and collaborators explored the adaptive significance of alternative life-history strategies, male and female mate selection, kin recognition, cannibalism, the femme-fatale effect, and many other behaviors. His work was beginning to draw the attention of the broader scientific medium with the *Manchester Guardian*, *Nature*'s "News and Views" section, as well as the *New Scientist* publishing accounts of his work. The publication in *Scientific American*'s April 1993 issue of his vision of the stickleback world attested to the international fame that he was developing.

We his colleagues miss terribly the opportunity to continue being part of Gerry's world. One was always guaranteed of stimulating hypotheses and exciting speculations when embarking on a discussion with Gerry. Small talk was not part of his vocabulary. Gerry was most appreciative of good science, honest people, straight talk, and being on time. He was absolutely honest in his dealings with people; one always knew exactly where one stood with Gerry. He did not suffer lightly the presence of those who did not place equal value on these attributes. His temper was sufficiently volcanic to ensure that transgressors rapidly learned the error of their ways.

Gerry is survived by his wife Anna-Lisa and his daughter Chèron. He is also survived by all those students and colleagues who valued his intensity, dedication, and honesty. He will be sorely missed.

Julian Dodson and Helga Guderley
Département de biologie,
Université Laval,
Ste-Foy, Québec

PERSPECTIVES IN COMPARATIVE ENDOCRINOLOGY

For the XII International Congress of Comparative Endocrinology, held in Toronto in May of 1993, more than 90 leaders in their various fields, speaking in 15 symposia, were invited to describe the important advances in their fields, and were challenged to provide the context so that these advances could be appreciated by the non-specialist. The great majority of those speakers have agreed to prepare their contributions for publication in *Perspectives in Comparative Endocrinology*.

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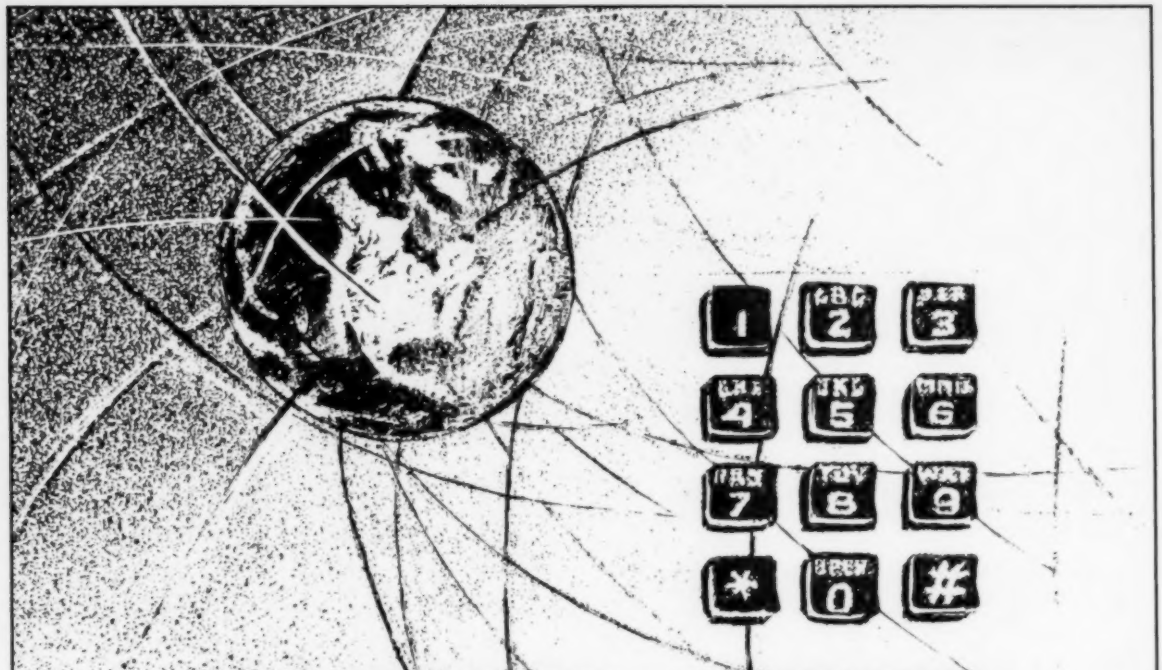

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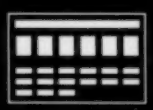

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